UNIVERSITY OF UDINE

FACULTY OF AGRICULTURE

Doctoral course in Food Science
Department of Food Science
(Course XXIV)

DOCTORAL DISSERTATION

Development and Characterization of HDPE/$CaCO_3$/TiO_2 Films for Food Packaging Applications

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ACADEMIC YEAR 2011-2012

A tutte le persone che
ho nel cuore,

TABLE OF CONTENTS

ABSTRACT

High density polyethylene (HDPE)/$CaCO_3$ films containing rutile TiO_2 were prepared via blown film extrusion process. Inorganic minerals embedded in films are used both to improve properties such as whiteness, impact resistance, tactile effect, and to reduce the content of the synthetic polymeric amount. In particular, TiO_2 applications are credited also to its strong UV-Vis light absorption leading to its well known photocatalytic activity. In order both to characterize the composite HDPE/$CaCO_3$/TiO_2 film, to evaluate the photocatalytic properties in the finished film and to develop an improved food packaging able to act as useful carrier of preservative substances, we proposed an investigation carried out using different approaches, such as ESEM-EDS, voltammetric, UV-Vis spectrophotometric, gas chromatographic and surface characterization. For the film containing 1% w/w of rutile TiO_2 the results rose in evidence both a homogeneous surfaces with an elemental distribution of titanium, calcium, carbon, oxygen and a photoinduced degradation activity displayed towards target organic compounds, such as methylene blue (MB) and 4-hydroxybenzoic acid (4-HBA), with production of CO_2 as the final degradation product. This photocatalytic property was confirmed by the slight conversion of film surface wettability. These results suggested that the blown film extrusion seems to be a good process to obtain composite food packaging films characterized by a homogeneous matrix. However, the direct inclusion of potassium sorbate before the blown extrusion process of the packaging film seemed to be not a suitable way to obtain a good preservative carrier. In fact, the preservative distribution in the resultant film was scarcely homogeneous, influencing the food packaging effectiveness. Real tests were performed on traditional food matrices, such as mozzarella and stracchino cheeses, using produced films as food packaging. Results were discussed and the profitable use of this photoactive film was proposed.

RIASSUNTO

Film flessibili per imballaggio alimentare costitutiti da polietilene ad alta densità (HDPE), carbonato di calcio ($CaCO_3$) e biossido di titanio (TiO_2) nella forma cristallina di rutile sono stati realizzati mediante il processo di estrusione definito *blown film*. I minerali inorganici contenuti nei film sono stati utilizzati per impartire sia importanti proprietà, come ad esempio la colorazione bianca, una maggiore resistenza meccanica e l'effetto tattile, sia per ridurre il contenuto di polimeri sintetici. In particolare, le applicazioni che riguardano il TiO_2 sono legate alla sua elevata capacità di assorbire la radiazione luminosa nell'intervallo UV-Vis ed alla sua correlata e ben nota attività fotocatalitica. Allo scopo di caratterizzare il film composito di HDPE/$CaCO_3$/TiO_2, di valutare le sue proprietà fotocatalitiche e di sviluppare un imballaggio alimentare in grado di veicolare sostanze attive capaci di estendere la vita commerciale dell'alimento confezionato, abbiamo proposto un'indagine eseguita mediante diversi approcci come l'ESEM-EDS, la voltammetria, la spettrofotometria nell'intervallo UV-Vis, la gas cromatografia e la caratterizzazione superficiale mediante la misura dell'angolo di contatto. I risultati riguardanti i film contenenti l'1% di TiO_2 nella forma cristallina di rutile hanno messo in evidenza sia superfici omogenee dove vi erano distribuiti elementi come titanio, calcio, carbonio, ossigeno, sia un'attività di degradazione fotoindotta a carico di molecole organiche usate come indicatori, tipo blu di metilene (MB) e l'acido 4-idrossibenzoico (4-HBA), che hanno portato alla produzione finale di anidride carbonica. Questa proprietà fotocatalitica è stata confermata anche dalla leggera conversione in termini di bagnabilità superficiale. Questi risultati hanno suggerito che l'estrusione mediante *blown film* sembra essere un processo in grado di produrre film caratterizzati da matrici omogenee. Tuttavia l'inclusione diretta di sorbato di potassio prima dell'estrusione si è rivelata un metodo poco utile per realizzare un film per imballaggio alimentare in grado di veicolare un conservante usato tradizionalmente come additivo. Infatti la distribuzione del sorbato di potassio nel film è risultata essere scarsamente omogenea, influenzando così l'efficacia dell'imballaggio. Sono state eseguite delle prove su alimenti tradizionali come mozzarella e stracchino che sono stati confezionati direttamente in linea produttiva nei materiali oggetto di studio. I risultati ottenuti sono stati discussi e l'utilizzo del materiale fotoattivo è stato proposto.

ABBREVATIONS AND SYMBOLS LIST

Allyl-isothiocyanate	AITC
Amplitude	τ_0
Calcium carbonate	$CaCO_3$
Complex shear modulus	G^*
Conduction band	CB
Coordination number	z
Cyclic voltammetry	CV
Deformation rate	$\dot{\gamma}$
Different pulse voltammetry	DPV
Energy band-gap	E_{bg}
Energy dispersive spectroscopy	EDS
Environmental scanning electone microscope	ESEM
Ethylene-vinyl acetate	EVA
Ethylene-vinyl alcohol	EVOH
Ethylene	C_2H_4
European Communities	EC
European Food Information Resource	EuroFIR
European Food Safety Authority	EFSA
European Union	EU
Frequency	ω
Gas chromatography-thermal conductivity detection	GC-TCD
Generally recognized as safe	GRAS
Glass transition temperature	T_g
Good Manufacturing Practice	GMP
Half-peak potential	$E_{p/2}$
$HDPE/CaCO_3/TiO_2$ composite film	OV
$HDPE/CaCO_3/TiO_2$/potassium sorbate composite film	OVA
Headspace-gas chromatography	HS-GC
High-density polyethylene	HDPE
High-performance liquid chromatography	HPLC
Hydrogen cation	H^+
Hydrogen peroxide	H_2O_2
Hydroxyl radical	$^{\bullet}OH$
Inductively coupled plasma-mass spectrometry	ICP-MS
Instantaneous potential	E_t
Lactic acid bacteria	LAB

Loss modulus	G''
Low-density polyethylene	LDPE
Linear low-density polyethylene	LLDPE
Linear sweep voltammetry	LSV
Maximum amplitude of deformation	γ_0
Melt temperature	T_m
Methylene blue	MB
Nitric acid	HNO_3
Organic substrate	RX
Oriented polypropylene	OPP
PE/PS/OV food tray	TOV
PE/PS/OVA food tray	TOVA
Peak current	i_p
Peak potential	E_p
Perhydroxy radical	$^{\bullet}OOH$
Phase angle	δ
Polyethylene	PE
Polypropylene	PP
Polystyrene	PS
Polyamide	PA
Polyether-ether-ketone copolymer	PEEK
Polyethylene teraphthalate	PET
Polyvinyl chloride	PVC
Polyvinyl acetate	PVA
Potassium sorbate	SP
Potential	E_i
Protected Designation of Origin	PDO
Protected Geographical Indication	PGI
Reference food packaging material	RIF
Relative humidity	R.H.
Scan (or sweep) rate	v
Shear strain	γ
Shear stress	τ
Storage modulus	G'
Strength of the network	A
Superoxide anion	$O^{\bullet}{}_2^{-}$
Surface tension of the liquid-vapour	γ_{LV}
Surface tension of the solid-liquid	γ_{SL}
Surface tension of the solid-vapour	γ_{SV}
Texture profile analysis	TPA
Tetrafluoromethoxyl polymer	TFM
Titanium dioxide	TiO_2
Ultraviolet light	UV
Ultrasound treatment	UT

Valence band	VB
Visible light	Vis
Water contact angle	θ
4-hydroxybenzoic acid	4-HBA
3,4-dihydroxybenzoic acid	3,4-DHBA

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In this opening chapter we provide an insight into the role, properties and innovation of food packaging materials and, in particular, polymer films. The food packaging concept is introduced with an emphasis on the equilibrium between packaging benefits and costs.

Background

Packaging is an essential component that affects virtually every industry. Each product, above all perishable items, needs a protection during its shelf-life and it is calculated that the 99.8% of beverages and foods are encased in some sort of packaging (Mahalik & Nambiar, 2010). Considering that the microbial and chemical contaminations are undesirable for food products, it is clear to notice that food packaging is used with the aim to prevent or impede these defilements (Hotchkiss, 1997). Thus, food packaging plays a significant role in the food supply chain and it is an integral part of both food processes. For these reasons, food and beverage companies are continually evolving with new food packaging technologies that enhance the quality of the products, prolong shelf-life, and positively impact the profitability of a product by reducing waste and spoilage (Restuccia et al., 2010).

Generally, a conventional food packaging is meant for mechanical supporting of food, protecting it from external influences such as heat, light, moisture, oxygen, off odours, insects, dust, and so on (Marsh & Bugusu, 2007). This principal function involves retardation of deterioration and safety maintenance of packaged food for a reasonable time. Performing the sole protection, it is evident that traditional food packaging has been conceived as an inert package with a passive role and minimum interaction between food and packaging. As a consequence, the extension of shelf-life is achieved using a combination of effective packaging with various strategies such as temperature and moisture control, drying, removal of oxygen or addition of chemicals (e.g., salt, sugar, natural acids, etc.) and preservatives (Robertson, 2006). However, some of these techniques cannot be applied to some minimally processed food such as fresh meats and ready-to-eat foodstuffs (Quintavalla & Vicini, 2002).

In the last decade, the wide demand for minimally processed, easily prepared and ready-to-eat food products, due to changes in retailing practices and consumers way of life, has posed major challenges for food safety and quality (Appendini & Hotchkiss, 2002; Coma, 2008). Recent food-borne microbial outbreaks are driving a search for innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness and safety. One option is to use new packaging concepts to provide an increased margin of safety and quality (Conte et al., 2007; Guillard et al., 2009). The most innovative development in the food packaging field has been the active packaging, a system able to provide deliberate interactions with the food or the headspace in order to extend the shelf-life and the maintenance of food safety, decreasing the addition of preservatives (Dainelli et al., 2008; Restuccia et al., 2010).

The first opportunity for active packaging to be used in Europe was offered by the Council Regulation No 1935/2004 of 27 October 2004 (Commission of the European Communities, 2004). Its general requirements have been recently integrated by Council Regulation (EC) No 450/2009 of 29 May 2009 (Commission of the European Communities, 2009) which establishes that the substances responsible for the active functions can either be contained in separate containers or directly be incorporated in the packaging material. Moreover, the Council Regulation (EC) No 1907/2006 of 18 December 2006 (Commission of the European Communities, 2006c) improved the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances, placing greater responsibility on industry to manage the risks from chemicals and to provide safety information on the substances used. One of the main reasons for adopting the Council Regulation (EC) No 1907/2006 was that a large number of substances have been manufactured and placed on the market in Europe for many years without sufficient information on the hazards that they posed to human health and the environment. As a consequence, these Regulations permitted a higher diffusion of active food packaging systems that actually can take several forms including (Appendini & Hotchkiss, 2002; Gutiérrez et al., 2009):

- addition of sachets or pads containing volatile agents into packages;
- incorporation of volatile and non-volatile agents directly into polymers;
- coating or adsorbing agents onto polymer surfaces;
- immobilization of agents to polymers by ion or covalent linkages;
- use of polymers with particular properties.

Above these assumptions, it is evident that active packaging can provide some functions, such as scavenging of oxygen, moisture or ethylene, emission of ethanol and antimicrobial activity, that do not exist in conventional packaging systems. As a consequence, this innovative technology permits to extend the shelf-life of the packaged foodstuffs, inhibiting the microbial growth and preserving their sensory properties (Buonocore et al., 2005).

Typicity and technological innovation: a successful controversy

The maintenance of sensory properties is particularly important in all foods where the decrease of typical sensorial characteristics cause the loss of food commercial value. This deterioration is often due to microbial growth and oxidation reactions, two different phenomena that occur on food surface (Guillard et al., 2009). Considering that minimally processed foodstuffs or traditional meals can not tolerate some methods of preserving foods, it is evident that an innovative food packaging technology can play an important role to control the degradation, above all for high value-added food products. Firstly because food packaging, such as an active packaging system, can concentrate its action both directly on the food surface and through the headspace around the food, then it allows to reduce the use of preservatives into food formulation (Quintavalla & Vicini, 2002). The latter is an important factor for foodstuffs where the absence of preservatives is preferred with the aim both to satisfy a consumer request and to respect a traditional food formulation (Davidson & Juneja, 1990). For example, traditional specialities possess some qualities, related to the natural origin of typical ingredients and the additives absence, that can influence sensory properties and consumer's expectations (Cayot, 2007). Representing a culture expression of a certain geographic area, these products, obtained by artisan preparation, are referred both to typical ingredients and an authentic production process which is commercially available for about 50 years (Bertozzi, 1998; Jordana, 2000; Gellynck & Kühne, 2008). Their definition and expression are promoted by the European Union (EU) through a project, called EuroFIR (European Food Information Resource) with the specific aim to distinguish the products considered traditional in food composition tables (Trichopoulou et al., 2007), and two directives, the Council Regulations (EC) No 509/06 and No 510/06 of 20 March 2006 (Commission of the European Communities, 2006a; Commission of the European Communities, 2006b), where many foods were covered and registered by EU as "Protected Designation of Origin (PDO)" or "Protected Geographical Indication (PGI)".

Inevitably, these regulations have increased the attention of food companies towards new convenience products that can evoke traditional taste and flavour, such as dairy (e.g. stracchino, mozzarella, ricotta cheeses) and bakery products, salami and so on (Kühne et al., 2010). Considering that these niche foodstuffs had a limited diffusion until 10 years ago, it is clear that the innovation becomes a strategic tool to achieve competitive advantage also for artisan foods, despite the apparential controversy between innovation and tradition (Gellynck et al., 2007; Jordana, 2000).

Costs and benefits of food packaging: a difficult equilibrium to reach

Every innovation, also the most important, has benefits and costs. The equilibrium between these two factors is very important and it drives the future success. Also in the field of food packaging exist benefits, such as the food quality and safety maintenance and the enhance of shelf-life, and costs, always joint to the raw material purchased and the complexity of the production process, that influence the diffusion of each food packaging solution (Vermeiren et al., 1999). Generally, most food packaging systems actually used are petroleum-based as these synthetic materials, characterized by low cost and large availability, provide a good barrier and mechanical properties. However, the use of these components is subjected to a progressive restriction in view of their not totally recyclable or biodegradable nature leading to environmentally harmful waste (Siracusa et al., 2008). Thus, biodegradable polymeric hosting matrices have been proposed during the last decade (Farris et al., 2009; Flores et al., 2010; Joo et al., 2011; Liu et al., 2004). Notwithstanding their environmental valuable attribute, bio-based polymers are affected by unsatisfactory dispersion stability, scarce mechanical properties and low filmable efficiency. Improvement of these properties could be achieved reducing the content of the polymeric amount by replacement with inorganic materials, thus also achieving better mechanical properties and flame retardancy (Hamming et al., 2009; Shah & Fuzail, 2006). Among the inorganic minerals used as fillers, calcium carbonate ($CaCO_3$) is a very low cost material with hydrophilic behaviour and good stability. In powder form (particle size ranging from 1 to 10 μm), it can be added in large amounts (20-60% w/w) with both the aims to reduce raw material costs and to improve properties such as whiteness, impact resistance, stability and tactile effect (Li et al., 2003; Tanniru & Misra, 2006). The latter is sought because it provides positive feelings that influence consumers' appeal toward a food product. Actually, food packaging acts as a "silent seller" because it may encourage consumer to buy regarding its appearance and shape (Kotler & Keller, 2006; Piergiovanni & Limbo, 2010a).

Packaging whiteness and brightness can also be improved by the addition of mineral oxides such as titanium dioxide (TiO_2) in small amounts ($< 5\%$ w/w). TiO_2 in its three main crystalline forms (i.e. rutile, anatase and brookite) has widespread applications not only in paints or plastics, but also in foods (i.e. used to whiten skinned milk), electronics circuits, sunscreens, environmental and purification treatment (Mohamed & Mkhalid, 2010). Generally, all these applications are credited also to its strong UV light absorption leading to its well known photocatalytic activity (Fujishima & Honda, 1972; Mills & Le Hunte, 1997; Srinivasan & Somasundaram, 2003; Zhao & Yang, 2003). This notwithstanding, until now the direct addition of TiO_2 powders in food packaging films has not been investigated and only coating, deposition or spraying applications were proposed.

In this PhD thesis, a food packaging film based on high density polyethylene (HDPE) resin added with inorganic minerals was developed and characterized. In particular, the PhD thesis was divided in 3 different parts. In the first, we focused our interests on the basic characterization of HDPE/$CaCO_3$/TiO_2 film in order to develop an improved food packaging able to act as useful carrier of preservative substances. In the second, the TiO_2 photocatalytic activity in the film was investigated, comparing the photocatalytic properties of whole film with the photocatalytic activity displayed by aqueous suspensions of commercial TiO_2 micro- and nano-powders. This investigation was carried out using voltammetric, spectrophotometric, gas chromatographic and water contact angle measurements. Then, in the last part, we proposed the evaluation of films effectiveness using two different real traditional food matrices, such as mozzarella and stracchino cheeses. Films characteristics and photocatalytic reaction mechanisms were discussed both to understand HDPE/$CaCO_3$/TiO_2 film behaviours and to increase the film performances for future perspectives.

PART I

Monolayer films for food packaging: production and characterization

I. GENERAL SECTION

I.1.1. Plastic polymers for food packaging: characteristics, uses and manufacture

I.1.1.1. Plastic polymers

By conventional definition, a polymer is a high-molar mass macromolecule made up of multiple equivalents of one or more repeating structural units, called monomers, linked together by covalent bonds (Flory, 1953). Although the term polymer is often taken to refer to plastics, it actually encompasses a large class of materials that can be divided into two categories (Jenkins & Harrington, 1991; Steinka et al., 2006):

- natural polymers, including cellulose, starch, lignin, rubber, latex and natural biopolymers such as proteins and nucleic acids;
- synthetic polymers, that are often referred to plastics such as polyethylene (PE), polypropylene (PP), polystyrene (PS), nylon, polyesters, etc.

For their wide range of properties, polymers play an essential role in everyday life. In particular, synthetics are the most used materials for food and non food applications. These petroleum-based polymers are obtained through a polymerization process consisting of chemical reactions in which a large number of monomers are joined sequentially, forming three-dimensional networks (Campbell, 1994). The characteristics of polymerization process permit the classification of polymers. In fact, if all atoms in the monomers are incorporated into the resultant polymer, it is called "addition polymer", while if some atoms are released into small molecules, such as water, the polymer is called "condensation polymer" (Young & Lovell, 1991). PE is a common example of addition polymer. It is based on a simple monomer such ethylene (C_2H_4) and its polymerization results in joining thousands unit of C_2H_4 together, forming a long chain.

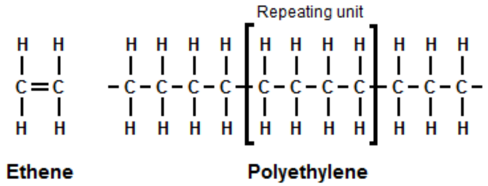


Figure 1. *An example of plastic addition polymer: polyethylene.*

Generally, the process of polymerization is driven by some factors, such as temperature, pressure, chemical nature of monomers and the presence of catalysts, that can influence the polymer properties. Different combinations of these factors can define some chemical-physical characteristics of the material, e.g., crystallinity, side-chain branching, molecular weight distribution, orientation etc., influencing its applications. For example, in plastic polymers the high reversible extensibility, due to both toughness and high viscosity, is an expression of property dependent on the chain structure, while the high permeability to fluids, compared with metals and glasses, is a further consequence of their organic structure (Birley, 1982; Robertson, 1993).

Independently from the polymerization conditions, plastic polymers are generally considered as materials:

- low cost;
- flowable and mouldable under certain conditions;
- chemically inert;
- are lightweight;
- do not support the growth of microorganisms;
- able to provide choices in respect of transparency, colour, heat sealing, heat resistance and barrier.

All these characteristics permit a large use of plastics in the food packaging sector because they provide a good barrier and mechanical properties for widespread applications, from deep frozen food storage to the high temperatures of retort sterilization and radiant heat (Kirwan & Strawbridge, 2003).

I.1.1.2. Use of plastics in food packaging

The packaging is one of the most important technological steps for food preservation since it provides a safe transport and an extended shelf-life using fewer chemical additives. These goals can be reached using materials able to provide both good barrier and commercial advantages. Most food packaging systems actually used are made of plastic polymers that are chosen for their low cost and large availability. Considering their basic characteristics, plastic polymers can be extruded and molded in order to obtain containers, components, monolayer flexible film or multilayer films. The latter system is achieved combining different plastic matrix through particular processes such as coextrusion, blending, lamination or coating (Kirwan & Strawbridge, 2003).

Nowadays, there are several specific examples of food packaging systems or components made by plastic polymers:

- rigid plastic containers such as bottles, jars, pots, tubs and trays;
- flexible plastic films in the form of bags, sachets, pouches and heat-sealable;
- plastics combined with paperboard in liquid packaging cartons;
- expanded or foamed plastic for uses where some form of insulation, rigidity and the ability to withstand compression is required;
- plastic lids and caps and the wadding used in such closures.

I.1.1.3. Types of plastic used in packaging

The choice of the food packaging material is commonly influenced by chemical-physical characteristics and storage conditions of the food. Clearly, food packaging systems have to satisfy safety and legal aspects, increasing their possible applications to different foodstuffs. For this reason, plastic raw material manufacturers have significant scope for tailoring their products to the requirements of the many applications for which plastics are used (Brennan & Day, 2006; Restuccia et al., 2010). Thus, there is a deep variety of plastic materials available with a wide range of properties, such as improved barrier, heat sealability, adhesion, strength and heat resistance.

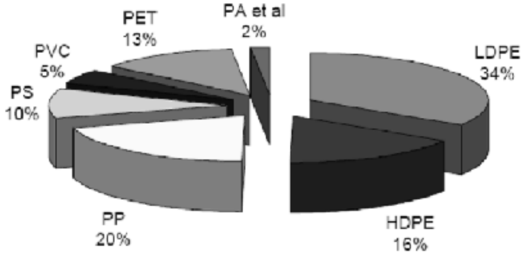


Figure 2. *The variety of plastic polymers used in the food packaging field (Piergiovanni & Limbo, 2010a).*

Actually, the most used plastic polymers in the European packaging field are PE, PP and PS: they are all thermoplastic and differ from thermosetting polymers (e.g. polyester, epoxy resin, polyamides) because they are characterized by the absence of cross-linking between polymer chains and, consequently, they can be softened repeatedly by heat and pressure to form different shapes. For the absence of cross-linking, thermoplastic polymers have higher recyclability than thermosetting plastics which are better suited for high-temperature applications and more brittle (Kirwan & Strawbridge, 2003).

The presence of cross-linking can influence much behaviour of plastic materials, not only the allowable temperature range in which the polymer can effectively operate. Together with molecular weight, stiffness of the molecular chain, intermolecular forces and side branching, cross-linking has effects on molecular mobility. Also environmental and loading conditions can act upon the molecular mobility and, indirectly, the glass transition temperature (T_g) which is largely depends both on chemical structure and molecular mobility (Ferry, 1980).

The glass transition is a phase change where a smooth increase in the viscosity of the amorphous solid without any pronounced change in the structure occur and the material is converted to a relatively hard and glassy state from a soft, elastic-plastic and rubbery state when the temperature is being cooled through its T_g. It does not apply only to amorphous noncrystallizable materials, but also to all solids that can not fully crystallize under supercooled conditions, such as semicrystalline polymers and amorphous metals. Considering that T_g is a direct measurement of molecular mobility, it is clear that below the T_g molecules are confined at the site with a very limited group or branch movement freedom, and their free volume is relatively small; consequently, whole molecules cannot move away from each other and polymers will show glass-like properties with lower permeability and higher brittleness. Instead, above the T_g molecules have much more freedom of movement and their free volume increases faster with the temperature: whole molecules can shift or slide away from each other and the material will present a rubber-like state (Gibbs & Di Marzio, 1958; Götze, 1990).

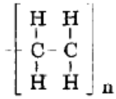
Basically, the polymers with longer molecular chains, more hydrogen bonds, polar or polarizable groups and cross-linking networks tend to have higher T_g than materials with symmetric molecular structures (Li, 2000). PE, PP and PS are three typical examples of semicrystalline thermoplastics with different glass transition temperatures (Tab. 1) due to their dissimilar chemical structures. Normally, PS is used in a glass state because its T_g is higher than room temperature, while PE and PP are often used in rubbery state resulting more chemically resistant and also opaque because their crystallites are larger than the wavelength of light (Kirwan & Strawbridge, 2003).

Table 1. *T_m and T_g of some plastic materials used in food packaging (Piergiovanni & Limbo, 2010a).*

Type of plastic	T_m (°C)	T_g (°C)
Polypropylene (PP)	176	-20
High density polyethylene (HDPE)	137	-125
Low density polyethylene (LDPE)	110	-25
Polystyrene (PS)	94	94
Polyamides (PA)	260	50
Polyethylene terephthalate (PET)	265	69
Polyvinyl chloride (PVC)	212	87

Based on the above assumptions, it is reasonable to consider that the behaviours of food packaging made of plastic polymers are influenced by some factors which have to be judged in order to avoid unwanted changes in the mechanical and physical properties of materials during their applications.
Following are general discussions of some commonly used food packaging polymers.

Polyethylene (PE)



PE is the most frequently used polymer in food packaging applications. It is structurally the simplest plastic because it is made only by addition polymerization of C_2H_4 gas. A range of low and high density resins are produced, depending on the polymerization conditions: high-density PE (HDPE), low-density PE (LDPE), and linear low density PE (LLDPE) are differing in density, chain branching, crystallinity and so on (Fig. 3). HDPE has a density around 0.94-0.96 g/cm^3. It is a linear polymer with relatively few side-chain branches and hence the macromolecules can fold and pack into an opaque, highly crystalline structure. Compared to LDPE, HDPE has a higher T_m (typically 137°C vs. 110°C), greater tensile strength and hardness, and better chemical resistance. LDPE has a density between 0.91 and 0.94 g/cm^3. It is a polymer with many long side-chain branches. The packaging film made from LDPE is soft, flexible, and stretchable with good clarity and heat sealability. LLDPE is a copolymer with many short side-chain branches. It has the same density, clarity and heat sealability of

LDPE, but it is preferred to LDPE because it presents the strength and toughness as well as HDPE. For these reasons, LLDPE has been replacing LDPE in many food packaging applications (Kirwan & Strawbridge, 2003).

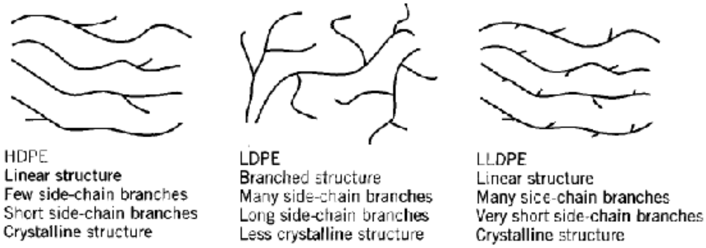
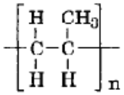


Figure 3. *Molecular arrangements of HDPE, LDPE, and LLDPE (Kirwan & Strawbridge, 2003).*

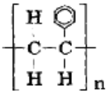
Polypropylene (PP)



$$\left[-\overset{\displaystyle H}{\underset{\displaystyle H}{C}}-\overset{\displaystyle CH_3}{\underset{\displaystyle H}{C}}- \right]_n$$

PP is a linear, crystalline polymer that has the lowest density (0.88-0.91 g/cm^3) among all major plastics. Compared to PE, PP has higher tensile strength, stiffness, and hardness. It has the T_m at about 176°C and hence it is more suitable for hot filling and retorting applications. Commercial PP films are available in oriented and unoriented forms. Orientation can be achieved by stretching the film either uniaxially or biaxially during the film-forming process. As a result, oriented PP film (OPP) has improved strength, stiffness and gas-barrier properties; however, it is not heat sealable. Unoriented PP film has excellent clarity, good dimensional stability, and good heat-seal strength. Beside films, other major applications for PP are containers and closures (Kirwan & Strawbridge, 2003).

Polystyrene (PS)



PS is an amorphous polymer that has excellent clarity. It is a clear and low-gas-barrier material with a relatively low T_m (94°C) and it can be readily thermoformed or injection molded into items such as food containers, cups, closures, and dishware. Because of its excellent clarity, PS film is often used as windows in paperboard boxes to display products, such as baked goods, pasta, pizza etc., that do not require a good gas barrier.

Expanded PS (EPS) of various bulk densities are manufactured by adding foaming agents in the extrusion process. EPS is used to make cups, plates, meat trays, clamshell containers, and egg cartons as well as protective packaging for shipping (Kirwan & Strawbridge, 2003).

Table 2. *Main characteristics of some common packaging plastics (Brody & Marsh, 1997; Piergiovanni & Limbo, 2010a).*

	HDPE	**LDPE**	**PP**	**PS**
Ultimate strength (MPa)	22-30	8-30	30-40	36-57
Elongation at break (%)	10	100-200	100	1
O_2 permeability*	2800	7000	2300	3800
WVTR**	5	15-25	4-10	100-155

*Unit in $cm^3\ 24h^{-1}\ m^{-2}\ bar^{-1}$; thickness: 25 µm
**Unit in $g\ m^{-2}\ 24h^{-1}$; 38°C, 90% RH

I.1.1.4. Manufacture of plastics packaging

As we have already seen, there are several examples of systems actually used in the field of food packaging. In order to obtain accessories, flexible or rigid packaging, the plastic raw material, also known as resin and usually supplied by the polymer manufacturer in the form of pellets, has to be converted in an extruder from a solid physical state to a molten phase.

During the extrusion process, the pellets are melted by a combination of high pressure, friction and externally applied heat. This is done by forcing the pellets along the barrel of an extruder using specially designed screw under controlled conditions that ensure the production of a homogeneous melt prior to extrusion (Kirwan & Strawbridge, 2003; Flores et al., 2010). A schematic representation of an extruder is shown in Fig. 4. Generally, the process begins by introducing the resin in the extruder: pellets are gravity fed from a top mounted hopper into the barrel of the extruder. Also if the formulation of the pellets could foresee small amounts of plasticizers, antioxidants and heat stabilizers able to facilitate the extrusion process, others additives such as colorants (master batch) or active substances (also in powder form) can be mixed with the resin prior to arriving at the hopper.

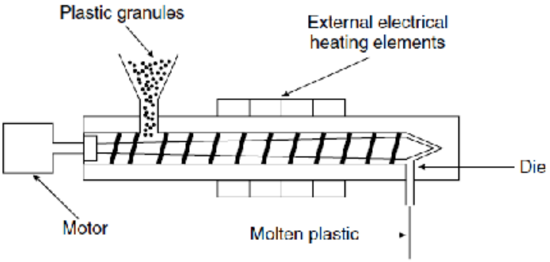


Figure 4. *A schematic representation of an extruder (Kirwan & Strawbridge, 2003).*

Then, the raw material enters through the feed throat and comes into contact with the screw. The rotating screw forces the plastic pellets forward into the barrel which is heated to the melt temperature of the molten plastic which depending on the polymer characteristics. A heating profile is set for the barrel in which more independent controlled heater zones gradually increase the temperature from the feeding zone to the die zone. This allows the plastic pellets to melt gradually, decreasing the risk of overheating which may cause polymer degradation. An extra heat is contributed by the intense pressure and friction taking place inside the barrel: in fact, if an extrusion line is running a certain material fast enough, the heaters can be shut off and the melt temperature maintained by pressure and friction alone inside the barrel. At the front of the barrel, the molten plastic leaves the screw and enters the die which gives the final product profile (Pearson, 1966).

There are two distinct methods of processing the molten plastic: cast film and blown film process (Agassant et al., 1991). In the cast film process, the molten plastic is extruded through a straight slot die onto a cooled cylinder, known as the chill roll (Fig. 5).

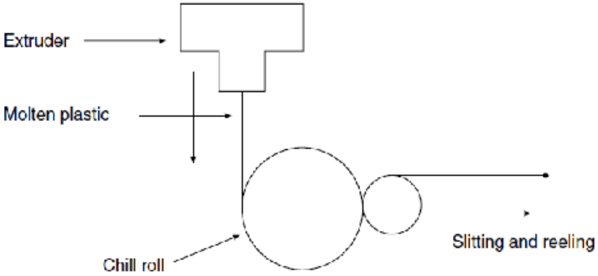


Figure 5. *A schematic representation of a cast film production (Kirwan & Strawbridge, 2003).*

In the blown film process, schematically represented in Fig. 6, the molten plastic is continuously extruded through a die in the form of a circular annulus. Around the circular die sits an air-ring where the compressed air is forced into the centre of the extruded circular profile, creating a stable film bubble (Flores et al., 2010; Pearson, 1966). This internal air pressure increase the ratio between the diameter of blown bubble and the diameter of the die, defined as blow-up ratio, decreasing the film thickness. Simultaneously, the guide frames above the die flatten the film which is subjected to tension in the axial direction. At the same time, an external air supplied from a concentric outer ring cools the film.

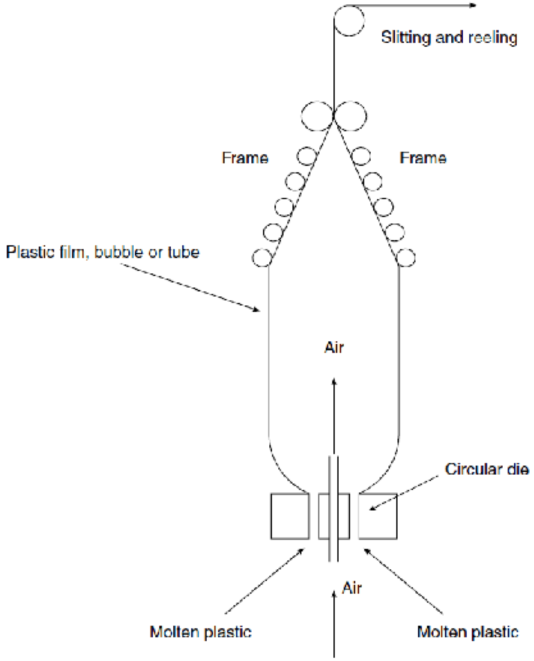


Figure 6. *A schematic representation of a blown film manufacture (Kirwan & Strawbridge, 2003).*

Anyway, in both the processes, the molten polymer is quickly chilled and solidified to produce a single layer film which is slit and reeled (Kirwan & Strawbridge, 2003). Generally, the extrusion is a process widely used to obtain films for food packaging. These are by definition less than 100 µm thick and principally used to wrap foodstuffs, overwrap packaging (e.g. single or groups of packs, palletised loads), make sachets, bags or they can also be combined in laminates with other plastics and materials (Pirkle & Braatz, 2011). Anyway, the properties of plastic films are strictly dependent both on the resin used and the extrusion method. For example, if the film is allowed to cool down slowly, then large crystals may be formed. This gives a hazy appearance due to the diffraction and scattering of incident light by the crystals. This behaviour is also available through the mix between pellets and active substances in powder form during extrusion process: in fact, if the size of the additive particle is too large or if they migrate to the surface, the film becomes hazy (Pearson, 1966).

The extrusion method and the characteristics of the process can also drive the film strength or improve higher barrier properties. Specifically, film can be stretched to realign or orient the molecules in both the machine direction and across the web in the transverse direction. Instead, with the blown film process orienting is already achieved by increasing the pressure inside the tube to create a bubble with a much larger diameter than original tube (Kirwan & Strawbridge, 2003).

I.1.2. Food packaging systems

I.1.2.1. The key concepts

Independently to its shape and characteristics, the most important objective of food packaging is in the efficient delivery of products to the consumer. To accomplish this goal, the package must serve the important functions of containing the food, protecting it from external physical, chemical, biological damages, and conveying product information (Hotchkiss, 1997). It is generally considered that food packaging systems consist of 3 different parts such as packaging materials, food products and the environment (Fig. 7).

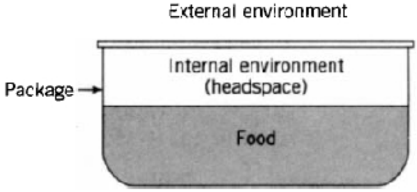


Figure 7. *Physical components of packaging system (Brody & Marsh, 1997).*

The latter is normally subdivided into internal and external environment: the internal, known as headspace, is directly in contact with the food, while the external depends on the storage and distribution conditions (Ahvenainen, 2003). Considering that the void volume of solid food products could be assumed as headspace, most food packaging systems represents either a package/food system or a package/headspace/food system. The first consists of a package in direct contact with food in a low viscosity, liquid or solid state. Wrapped cheese or aseptic meat packages are two examples of this system where the diffusion between the packaging and the food, partitioning at the interface, is the main migration phenomenon involved. Contrarily, in a package/headspace/food system such as flexible packages, bottles, cans and cups, the evaporation or equilibrated distribution of a substance among the packaging material, headspace and food is considered as a part of main migration mechanism. It involves principally volatile substances that can migrate through the headspace, while non-volatile substances can only migrate through the contact area between package and food (Han, 2003).

Above these assumptions, it is evident that an inert food packaging does not exist. In particular, a large number of plastic polymers used in food packaging applications contain small amounts of additives, such as plasticizers, lubricants, slip agents, antioxidants and heat stabilizers (Hotchkiss, 1997). These additives are used to facilitate the extrusion process or to impart desirable properties, but in certain conditions (e.g. storage temperature, light exposition, chemical nature of food, etc.) they can migrate into food. This phenomenon is an important source of health hazard subject to specifics international regulations (Dainelli et al., 2008; Restuccia et al., 2010). Then, food packaging has to be chosen for specific technical applications taking the specific needs in distribution and storage with the aim to protect the food in such a way that health is not endangered and that the quality is maintained.

I.1.2.2. Food contact and barrier properties: the issues

Many Authors (Heckman, 2007; Lagaron et al., 2005; Marsh & Bugusu, 2007, Piergiovanni & Limbo, 2010a) have shown that food quality depends also on the possible interactions between food and packaging. The result may be that detrimental organoleptic and other changes occur, which may be caused by:

- migration of additives, residues and monomer molecules from packaging into the food;

- permeation of gases, vapours and permeant molecules from the environment into the pack headspace and vice versa;
- sorption of volatile flavour compounds and lipids into the packaging.

In this first part of our work, we discuss only the issues caused by migration phenomena in terms of legal safety aspects.

I.1.2.3. Migration: basic concepts and legal issues

When food products are packaged, the food could be in direct contact with the inside surface of the packaging. Migration is possible for interaction between the food and the packaging to occur and for components of the packaging to be absorbed by, or react with, the food. In the case of plastic materials, this may involve the basic polymer, and even if this is non-reactive with respect to the ingredients of the food, it is possible that coatings and additives of food packaging may interact with the food. It is therefore essential that the plastic material and associated additives are approved for direct food contact.

To ensure that adequate product safety procedures are carried out, many countries have regulations to maintain safety with respect to plastics in contact with food. In the European Union, the Council Regulation (EC) No 10/2011 of 14 January 2011 (Commission of the European Communities, 2011) is part of the legislative framework covered by Council Regulation (EC) No 1935/2004 of 27 October 2004 (Commission of the European Communities, 2004) on materials and articles intended to come into contact with food: the aim is to ensure that they are manufactured in accordance with good practice and that they do not transfer any constituents into food. It includes definitions and migration limits with the aim to reduce the use of end residues associated with specific substances. A migration limit is either defined in terms of weight released per unit area, e.g. 10 mg dm^{-2}, or where this is either not feasible, e.g., for caps, gaskets or where the volume of the container is from 0.5 to 10 L, the limit is 60 mg kg^{-1} of the foodstuff concerned.

In terms of migration limits, the Council Regulation (EC) No 10/2011 repeals also the Directive 82/711/EEC (implemented with Directive 85/572/EEC and other amendments, i.e. Directive 93/8/EEC and Directive 97/48/EC). In fact, the new Regulation re-sets rules for migration testing using specified food simulants:

- simulant A: 10% v/v ethanol for hydrophilic products with alcoholic content $\leq$ 10%;

- simulant B: 3% w/v acetic acid for acidic foods (pH < 4.5);
- simulant C: 20% v/v ethanol for hydrophilic products with alcoholic content ≤ 20%;
- simulant D1: 50% v/v ethanol for food products such as milk, yogurt, cream;
- simulant D2: rectified olive oil for fatty/oily foods;
- simulant E: 2,6-difenil-p-fenilene polyoxide (particle size 60-80 mesh; porous size 200 nm) for dry food products.

Analytical and measurement techniques have been developed to identify and quantify the simulants migration. These test procedures are also used, together with sensory testing panels, to evaluate plastic materials and packaging in production and use (Frank et al., 2001).

I.1.3. Technological evolution of food packaging systems

I.1.3.1. A new concept of food packaging

Until few years ago, the concept of food packaging was based on the sole passive mechanical protection of solid and non-solid food from external influences. In fact, the packaging was considered only a barrier that had to be as inert as possible (Appendini & Hotchkiss, 2002). In these last years, the wide demand for minimally processed, easily prepared and ready-to-eat food products, due to changes in retailing practices and consumers life-style, has posed major challenges for food safety and quality (Coma, 2008; Robertson, 2006). Recent food-borne microbial outbreaks have driven a search for innovative ways to inhibit microbial growth in foods (Conte et al., 2007; Guillard et al., 2009). Traditionally, there are different methods, such as thermal processing, drying, irradiation, addition of antimicrobial agents and so on, able to preserve foods. Unfortunately, some of these techniques can not be applied to specific food products such as fresh meats, high-value added and ready-to-eat food products (Quintavalla & Vicini, 2002).

New concepts of food packaging offer several solutions for extending shelf-life or maintain food quality and safety. In these terms, the most innovative development in the food packaging field has been the active packaging, a system able to provide deliberate interactions with the food or the headspace (Dainelli et al., 2008; Restuccia et al., 2010). However, a limited regulation has caused the lack of penetration of active packaging in the European market in comparison to other countries, such as Japan, USA and Australia, where more flexible regulations have permitted in past years technological innovations.

The first opportunity for active packaging to be used in Europe was offered by the Council Regulation No 1935/2004 of 27 October 2004 (Commission of the European Communities, 2004). This Regulation has repealed the old legislation with the purpose to assure a high level of human health protection, setting the Framework for the European Food Safety Agency (EFSA) evaluation process. It authorizes the use of "active food contact materials and articles" that are defined as materials that are intended to extend the shelf-life or to maintain the condition of packaged foods releasing or absorbing substances into and from the packaged food or from the headspace. These materials have not to transfer their constituents to food in any quantity that could endanger human health or bring about any organoleptic change of the food and their manufacture has to be in accordance with Good Manufacturing Practice (GMP). Releasing systems are allowed to change the composition of the food without masking spoilage, providing that the released substance is an authorized compound. All passive parts are also subject to pre-existing European and national food contact material regulations under the principle of mutual recognition. Finally, labelling should comply with the food additive directive; moreover the release or absorption of substances should not mislead the consumer.

General requirements stated in Council Regulation (EC) No 1935/2004 for the safe use of active packaging have been recently integrated by Council Regulation (EC) No 450/2009 of 29 May 2009 (Commission of the European Communities, 2009). It establishes that the substances responsible for the active functions can either be contained in separate containers, such as small sachets, or directly be incorporated in the packaging material. These active components may release or absorb substances and not be carcinogenic. They have to be listed into the community list of authorised substances established by EFSA and must comply with the food additives legislation (Council Regulation (EC) No 1333/2008, Commission of the European Communities, 2008). The Council Regulation (EC) No 450/2009 introduces also the concept of functional barrier. It consisting of one or more layers of food-contact that preventing the migration of substances in amounts which could endanger human health. Consequently, these active substances do not need a safety evaluation. Finally, this Regulation justifies the use of nanotechnologies, assessing their application case-by-case and establishing a maximum migration limit at 0.01 mg kg^{-1}.

I.1.3.2. Active food packaging

Considering that food deterioration is often due to microbial growth and oxidation reactions, two different phenomena that occur on food surface, it is evident that active packaging can play an important role to control the degradation because its action is achieved directly on the food surface (Guillard et al., 2009). In order to extend food shelf-life, preservatives are widely added at the quantity required to control the degradation phenomena. However, the addition of preservatives into the food formulations may result in partial inactivation of the active substances by product constituents, having only limited effect on the surface. Food packaging can be used as carrier of preservatives or other active substances, concentrating them at the food surface where the protection is needed. That means that only very small amount of additives into the food formulation is required (Quintavalla & Vicini, 2002).

Based on these assumptions, it is clear that active packaging can provide several functions that do not exist in conventional packaging systems. These functions may include scavenging of oxygen, moisture or ethylene, emission of ethanol or flavours, and antimicrobial activity (Lim, 2011; Ozdemir & Floros, 2004; Vermeiren et al., 1999).

Table 3. *Examples of active packaging applications for use within the food industry (Lim, 2011).*

Absorbing/scavenging properties	Oxygen, carbon dioxide, moisture, ethylene, flavours, taints, UV light
Releasing/emitting properties	Ethanol, carbon dioxide, antioxidants, preservatives, sulfur dioxide, flavours, pesticides
Removing properties	Catalysing food component removal: lactose, cholesterol
Temperature control	Insulating materials, self-heating and self-cooling packaging, microwave susceptors and modifiers
Microbial and quality control	Surface-treated packaging materials

The principles behind active packaging are based either on the intrinsic properties of the polymer used as packaging material itself or on the introduction, by inclusion, entrapment, etc., of specific substances inside the polymer. The intrinsic properties of the polymer can give rise to the active function both through the intentional grafting of an active group and the introduction of an active monomer inside the polymer chain. An active agent can be incorporated inside the packaging material or onto its surface, in multilayer structures or in particular elements associated with the packaging such as sachets, labels or bottle caps. The nature of the active agents that can be added is very different (e.g., organic acids, enzymes, bacteriocins, natural extracts, ions, ethanol, and so on) as well as the nature of the materials into which they are included such as papers, plastics, metals or combinations of these materials (Ahvenainen, 2003; Lim, 2011). Generally, an active packaging can be classified into two main types (Fig. 8):

- non-migratory active packaging, that acting without intentional migration of active agents from the packaging into the food. The most well known examples are moisture absorbers, mostly based on the adsorption of water by a zeolite, cellulose and their derivatives. The tendency is to introduce the absorbing substances inside the packaging material in order to make the active system invisible for the consumer.

  Other well known systems are oxygen absorbers, mostly based on iron oxidation but they can also be based on ascorbic acid or catechol oxidation, on enzymatic catalysis as well as on many other reactions. The development of oxygen scavenging systems was first based on self-adhesive labels, others adhesive devices or loose sachets to be included in the packaging with the food.

  Another example of non-migratory packaging is the antimicrobial packaging based on the entrapment in a silicate network of silver ions, a widely marketed antimicrobial agent. Nevertheless, even food contact antimicrobial systems with an assumed non-intended migration are known to exhibit some degree of migration (Fernandez-Saiz et al., 2006; Goddard et al., 2007; Fernandes et al., 2008);
- active releasing packaging allowing a controlled migration of non-volatile agents or an emission of volatile compounds in the atmosphere surrounding the food. One of the best-known products is an ethanol releaser sachet which is able to slow down mould growth and thus increasing the shelf-life of bakery products.

  Another example is a plastic film containing allyl-isothiocyanate (AITC), a strong antimicrobial. AITC is entrapped in cyclodextrins for protecting the volatile active agent from being thermally degraded during extrusion. When exposed to high moisture conditions after the packaging of the food product, cyclodextrins have the ability to change in structure and to release the antimicrobial agent in the atmosphere surrounding the food (Lee, 2005).

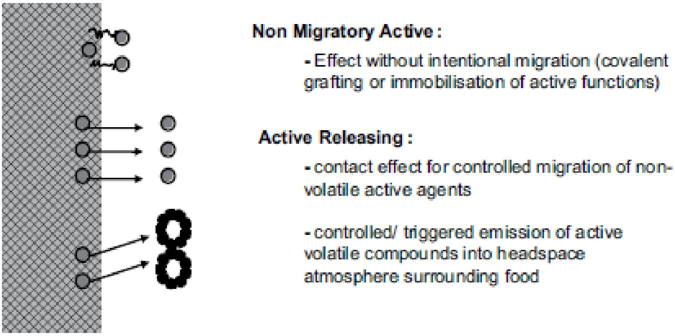


Figure 8. *Scheme of the two different types of active packaging classified as a function of intentional or unintentional migrations (Dainelli et al., 2008).*

I.1.3.3. The use of potassium sorbate as active substance

In order to reduce, inhibit or retard the growth of microorganisms, molds and yeasts that may be present at the food surface, three forms of active packaging have predominated: oxygen absorbers, moisture absorbers and ethanol vapour generators (Restuccia et al., 2010). However, all these successful commercial applications exploited the use of sachets that are enclosed or attached to the interior of the package.

An interesting alternative to these forms has involved the incorporation of active agents directly into the packaging materials (Gutiérrez et al., 2009). The concept of antimicrobial polymeric material was introduced for the first time in the biomedical science field (Endo et al., 1987). The incorporation of chemical preservatives or antimicrobial agents into packaging has demonstrated to enhance microbial safety because the active compounds can be effectively released to the foodstuffs (Han, 2000). The number of recently published articles suggests that studies on antimicrobial packaging for food applications have more than doubled in the past 5 years. GRAS (Generally Recognized as Safe), non-GRAS and natural substances have been incorporated into paper, thermoplastics and thermosets, evaluating their effectiveness against pathogenic microorganisms and spoilage organisms growth. Research has been performed using, for example, α-tocopherol into a HDPE/ethylene vinyl alcohol (EVA)/LDPE film (Granda-Restrepo et al., 2009), a dehydroacetic acid sodium salt incorporated into a HDPE film (Zema et al., 2010), a lysozyme immobilized into a polyvinalcohol (PVOH) film (Conte et al., 2007), α- and β-cyclodextrins encapsulated in polylactide-co-polycaprolactone films (Plackett & Ghambari-Siahkali, 2007), an Enterocin 416K1 incorporated into polymeric films (Iseppi et al., 2008), an antimicrobial peptide coated on a HDPE film (Miltz et al., 2006) and some natural extracts with antimicrobial properties, such as thymol, lemon extract, cinnamon and rosemary essential oil, incorporated into different polymeric matrices (Del Nobile et al., 2009; Nerín et al., 2008; Rodríguez et al., 2008). Thus, several approaches have been proposed but only a few of them have become commercially available because all thermal polymer processing methods used for commercial production may create some damage to heat-sensitive active substances (Gutiérrez et al., 2009; Han et al., 2007).

Meanwhile, active agents with highest melt temperature have been investigated by many Authors. Among these agents, potassium sorbate (SP) is commonly used in research papers. This potassium salt of sorbic acid is the most used form available for food systems because it has good thermal stability (T_m at about 270°C), high water solubility and neutral flavour (Sayanjali et al., 2011). To achieve a good preservative effect, it is important that the molecule dissociate or convert into sorbic acid.

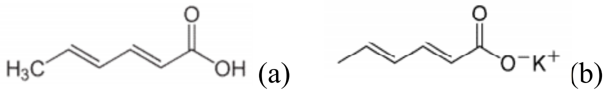


Figure 9. *Chemical formulae of sorbic acid (a) and potassium sorbate (b).*

The amount of dissociation is directly affected by the pH of the food system and it is highest at low pH levels (Sauer, 1977). However, SP exhibit the greatest activity against yeasts and molds than others food additives, such as propionates, benzoates and so on, inhibiting the growth of several spoilage organism also at about 6.5 of pH value (Arslan et al., 2009; Liewen & Marth, 1985; Sofos & Busta, 1981; Stratford & Anslow, 1996).

In literature, SP is often proposed as active agent in coating or drying applications (Choi et al., 2005; Jipa et al., 2012; Sayanjali et al., 2011; Uz & Altinkaya, 2011), while its incorporation through extrusion process is achieved by a few Authors (Flores et al., 2010; Han, 2000).

I.1.4. Wetting properties of food packaging

I.1.4.1. The importance of wettability evaluation

Plastic films for food packaging are widely utilized in the food industry due to their well known flexibility, light weight, barrier properties, resistance to breaking and cost effectiveness (Kirwan & Strawbridge, 2003). For production of new blend films with better characteristics, the knowledge of wetting properties represents a key parameter in order to enhance their aptitude to interact with the food, to adhere to other plastic layers, to be sealed and to promote their printability. In fact, these abilities are primarily influenced by surface wettability of films which is dependent upon the degree of contact achieved between a plastic surface and a wetting liquid and the magnitude of the intermolecular forces involved (Karbowiak et al., 2006a; Meiron & Saguy, 2007). Wettability is generally evaluated from contact angle measurements. As the contact angle between the test liquid and the film increases, the potential for the two to interact decreases (Mieke et al., 2002).

I.1.4.2. Thermodynamic definition of contact angle

Contact angle as a wetting phenomenon was originally defined in the early 1800 for solid, non-porous, non-absorbent surfaces for which the contact angle was taken at equilibrium. In 1805, Young described a relationship involving the surface tension at a point of the three-phase contact line between a smooth, rigid, solid phase S, a liquid L, and its vapor V:

$$\gamma LV \cdot \cos\theta = \gamma SV - \gamma SL$$

where γLV, γSV and γSL are the surface tensions of the liquid-vapour, solid-vapour and solid-liquid interfaces and θ is the contact angle (Young, 1805).

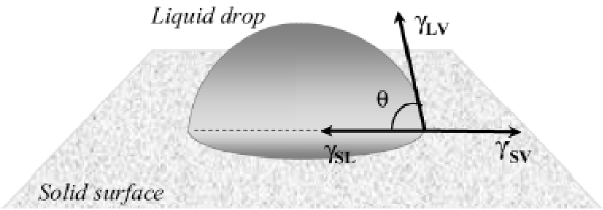


Figure 10. *Contact angle of a liquid drop on a solid surface and representation of surface tensions at the three-phase contact point (Karbowiak et al., 2006a).*

As presented on Fig. 10, the contact angle θ is basically defined as the angle between the solid surface and a tangent drawn on the drop surface, which passes through the triple-point of atmosphere-liquid-solid (Zisman, 1964). The phenomenon of spreading of the liquid over the surface, as measured by the contact angle, is then dependant on the relative magnitude of cohesive and adhesive molecular forces that exist respectively within the liquid and between the liquid and the solid. The value of the water contact angle θ with water indicates how hydrophobic the surface is. In theory, this contact angle may be from 0° up to 180°, that is to say from complete spreading of the liquid onto the solid surface up to the unrealistic limit of absolutely no wetting. Practically, a large water contact angle represents a hydrophobic surface, whereas a small water contact angle is related to a hydrophilic surface. The quantitative definition of the relative terms hydrophobic and hydrophilic surfaces has been done for surfaces exhibiting a water contact angle $\theta > 65°$ and $\theta < 65°$, respectively (Karbowiak et al., 2006a; Vogler, 1998).

The evaluation of surface wettability through this technique of surface forces measurement remains at present the simplest and most accurate method for quantifying the surface affinity for water or other test liquids. Anyway, various methods are used for measuring contact angle on different types of substrates. The methods are chosen considering the nature of substrate surface and the information to obtain by contact angle measurements (Karbowiak et al., 2006a).

I.1.4.3. Measurement of contact angle: the sessile drop method

For smooth surfaces, the most frequently applied approach for quantifying the contact angle of plastic materials is the optical method of sessile drop (Meiron & Saguy, 2007). In this static method, the sessile drop is produced before the measurement and it is assumed to be in complete mechanical equilibrium even if the value of θ, as commonly measured, is not the true equilibrium due to experimental conditions and tested surfaces that deviate from ideality. After the drop deposition on the film surface, a video acquisition of a magnified image of the drop profile is conveyed to a computer via a CCD camera, which enables to quantify changes in droplet shape recorded as digital images over time. Then, the magnified image is processed and a curve from a theoretical meridian drop profile is fitted, measuring contact angle between the baseline of the drop and the tangent at the drop boundary (Karbowiak et al., 2006b). The contact angles are determined by the analysis of the grey level values of the image pixels, using a software able to calculate the root of the secondary derivative of the brightness levels to receive the point of greatest changes of brightness. The found drop shape is adapted to fit a mathematical model which is then used to calculate the contact angle. There are various methods of calculating the contact angle that differ in the mathematical model used for analyzing the drop shape. The most used methods are the Young-Laplace fitting and the Tangent 1 fitting. The first can evaluate the complete drop contour, including a correction which takes into account the fact that it is not just interfacial effects which produce the drop shape, but that the drop is also distorted by the weight of the liquid it contains. Also the interfacial tension can be determined, but the calculation is only reliable for contact angle above 30°. Contrarily, Tangent 1 method fit the complete profile of sessile drop using a general conic section equation. Its derivative at the intersection point of the contour line with the baseline gives the slope at the 3-phase contact point and therefore the contact angle (Cheng et al., 1990; Page et al., 2000; Rotenberg et al., 1983; Schultz & Nardin, 1994).

I. EXPERIMENTAL SECTION

I.2. MATERIALS AND METHODS

I.2.1. Films manufacture

I.2.1.1. Materials

The raw thermoplastic composite material in the form of pellets (3-5 mm of diameter) was a 50% w/w chalk-filled compound based on high density polyethylene (HDPE) resin added with inorganic minerals, such as calcium carbonate ($CaCO_3$) and a small amount of titanium dioxide (TiO_2) in the form of rutile micropowder (0.2-0.3 μm). Potassium sorbate (≥ 99% w/w purity) in micropowder form (0.6-0.8 μm) was purchased from Aldrich (Sigma-Aldrich, Seelze, Germany). All films manufacture was carried out in industrial plant (Arcadia S.r.l., Udine, Italy).

I.2.1.2. Manufacture of basic films

The basic films (OV) were prepared through a blown film extrusion process, using a pellet containing $HDPE/CaCO_3/TiO_2$ in the w/w ratio of 49:50:1%, respectively. The raw thermoplastic composite pellets were gravity fed from a top mounted hopper into the barrel of a MAM extruder, model ES/KB 80 (CMG, Varese, Italy), heated from 150 to 190°C in which a single rotating screw forces the pellets forward. The molten material was pulled toward a MAM circular die, model CR350 (CMG, Varese, Italy), where an air compressed outlet forced the molten plastic to expand into a stable film bubble characterized by a blow-up ratio of 1:10. Finally, the bubble was flatted by some guide frames into a double layer film that will be slit and reel. All the system was automatically controlled to produce a single layer film with a thickness of about 40 μm.

I.2.1.3. Manufacture of active films

The active films (OVA) were prepared through a two-step process. The first step was consisted of a manually mixing of the same $HDPE/CaCO_3/TiO_2$ pellets used for OV films manufacture with the micropowder of potassium sorbate (SP). This batch was then automatically blended with a second batch of $HDPE/CaCO_3/TiO_2$ pellets by using the top mounded hopper in order to perform a batch ratio of about 10:90 w/w. Finally, the active films OVA were obtained by feeding the mix pellets-SP micropowder into the MAM single screw extruder. All the parameters adopted for OVA films, such as barrel zone temperature profile, rotating screw rate and so on, were the same of OV films. Consequently, the resultant monolayer OVA films had a thickness of about 40 µm with a SP concentration of 7 mg for every g of pellet used (7 mg/g w/w). The SP concentration was chosen in order to maximize the presence of the active substance, maintaining the blown film process workability at the same time.

I.2.2. Materials characterization

The materials characterization was carried out in the laboratories of the Department of Food Science (University of Udine).

I.2.2.1. Colour measurements

The colour measurements were performed by means of a Minolta colorimeter, model CR-200 (Konica Minolta, Osaka, Japan), using a 1.5 cm diameter aperture. In particular, OV and OVA film disks of an appropriate diameter were rested on a white colour tile standard ($L^* = 96.90$, $a^* = 0.14$, $b^* = 1.98$) according to Trezza & Krochta (2000) and the exposed area was sufficiently great with regard to the illuminated area to avoid any light trapping effect. Colour was expressed as CIELab coordinates (CIE standard 6774K): in this system, L^* represents the colour lightness on a 0-100 point scale from black to white; a^* is the position between red (+) and green (-); b^* is the position between yellow (+) and blue (-). All data shown are the average value of ten replicates.

I.2.2.2. The evaluation of pellet total ash content

The total ash content in pellet was evaluated according to ISO 3451-1 test method. Pellets (4 g) were flamed over a burner prior to being ashed in a muffle furnace (900 ± 10°C). After incineration, the total ash content was evaluated by measuring the weight of the samples by means of a RADWAG micro-balance, model AS 220/C/2 (RADWAG, Radom, Poland), with an accuracy of 0.0001 g. All data shown are the average value of three replicates.

I.2.2.3. pH measurement

Pellets (1 g) were added to 20 mL of high purity deionized water ($R > 18 M\Omega cm^{-1}$, $0.055\ \mu S cm^{-1}$, TOC < 5 ppb) obtained with a Milli-Q Integral system (Millipore Co., Billerica, MA, USA). In a same volume of high purity deionized water also the prepared films (OV/OVA, with size 0.0038 cm×180 cm^2), cut in small pieces (0.5×2 cm), were added. After 10 minutes at ambient temperature, under moderate stirring, the pH was measured using a digital pH metre Hanna pH301 (Hanna Instruments, Milan, Italy). The time of stirring was evaluated by monitoring the pH of the solution until an equilibrium value was reached. All data shown are the average value of ten replicates.

I.2.2.4. Moisture determination

Pellets (2 g) and the produced films cut in small pieces (2×2 cm) were dried in an oven at 110°C until reaching a constant weight. Then, the sample was weighed using a RADWAG micro-balance, model AS 220/C/2 (RADWAG, Radom, Poland) with an accuracy of 0.0001 g, and the results were expressed on a w/w dry basis. All data shown are the average value of three replicates.

I.2.2.5. Morphological characterization of pellet and films surfaces

Pellet and films surfaces were observed using an Environmental Scanning Electron Microscopy (ESEM), model XL 30 (Philips, Amsterdam, The Netherlands). In particular, some pellets and small films strips (5×3 mm) were fixed on the support using double-sided adhesive tape. EDS compositional maps of pellet and film surfaces were performed by dispersion spectroscopic analyses (ESEM-EDS) at an acceleration voltage of 20 kV.

I.2.2.6. Surface wettability and hydrophobicity of produced films

The surface hydrophobicity as the wettability of produced films were evaluated from contact angle measurements using a goniometer (Kruss GmbH, Hamburg, Germany) equipped with an image analysis software (Drop Shape Analysis, Kruss GmbH, Hamburg, Germany). A droplet (3 μL) of high purity deionized water (R > 18M$\Omega$$cm^{-1}$, 0.055 μS$cm^{-1}$, TOC < 5 ppb) was deposited with a precision syringe on the film surface. The sessile drop method was used and the water contact angle θ was obtained as function of time (0 and 60 s). Surface properties of OV and OVA films were compared with them exhibited by other food packaging films, such as HDPE and PP, kindly delivered by Poliflex (Poliflex S.r.l., Brescia, Italy). Ten measurements per film surface were carried out. All films were pre-conditioned in a chamber under the same environmental conditions to avoid interferences due to competing moisture exchange at the surface around the droplet.

I.2.3. High-performance liquid chromatography (HPLC) active substance assay

With the proposed model that follows, we present a method that try to provide accurate results for SP concentration and distribution in OVA film. The HPLC assay was carried out in the laboratories of the Department of Food Science (University of Udine).

I.2.3.1. Materials

Ammonium acetate (99.9% w/w purity) was purchased from Fluka (Fluka AG, Buchs, Switzerland). Glacial acetic acid and HPLC-grade acetonitrile were obtained from Carlo Erba (Carlo Erba Reagenti, Milan, Italy). Potassium sorbate ($\geq$ 99% w/w purity) was purchased from Aldrich (Sigma-Aldrich, Seelze, Germany). High purity deionized water ($R > 18 M\Omega cm^{-1}$, 0.055 $\mu S cm^{-1}$, TOC < 5 ppb) was obtained with a Milli-Q Integral system (Millipore Co., Billerica, MA, USA).

The ultrasound treatment (UT) was performed using a VWR Ultrasonic Cleaner 900 D (VWR International, Milan, Italy) at 60°C for 2 h with an ultrasonic intensity of 600 W/cm^2 and a frequency of 45 kHz. A high-performance liquid chromatograph from JASCO (JASCO Inc., Easton, PA, USA) was used equipped as follows: 880-PU pump, 875-UV photodiode array detector and 880-02 unit ternary gradient.

I.2.3.2. Potassium sorbate extraction

A random part (2500 cm^2) of the produced OVA film was cut in five pieces (2×4 cm), as shown in Fig. 11.

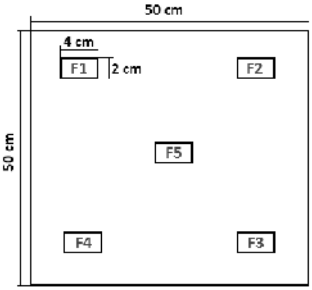


Figure 11. *Schematic representation of the pieces distribution used for HPLC assay.*

Every piece was cut again (0.5×1 cm) and put into a plain test tube with 10 mL of high purity deionized water. Then, all closed plain test tubes (F1, F2, F3, F4, F5) were subjected to UT. The time of UT application was evaluated by monitoring, by means of an HPLC, the SP concentration in the plain test tube aqueous solution until an equilibrium value was reached.

I.2.3.3. Mobile phase preparation for HPLC assay

The mobile phase consisted of 70% ammonium acetate buffer with 30% HPLC-grade acetonitrile and was prepared in two steps. Firstly, 0.23 g of ammonium acetate was dissolved in 700 mL of high purity deionized water. To this solution were added approximately 0.5 mL of glacial acetic acid and the pH adjusted to 4.2. The buffer solution was then transferred to 1 L volumetric flask, brought to volume and filtered through a 0.22 µm Durapore filter (Millipore Co., Billerica, MA, USA). Then, 700 mL of the acetate buffer solution were mixed with 300 mL of HPLC-grade acetonitrile. This was degassed and used for standard dilution, sample dilution and the HPLC mobile phase. The mobile phase was prepared according to Pylypiw & Grether (2000).

I.2.3.4. Standard preparation for HPLC assay

Potassium sorbate (1 mg) was added to a 100 mL volumetric flask and brought to volume with high purity deionized water. Dilutions of the stock solutions were made into mobile phase, by addition of 0.1, 0.25, 0.5, 1.0, 2.5, 5, 8 mL of stock to a total volume of 10 mL mobile phase to yield 0.1, 0.25, 0.5, 1.0, 2.5, 5, 8 mg/L calibration standards, respectively. Standard solutions were not filtered prior to HPLC analyses.

I.2.3.5. Sample preparation for HPLC assay

Pieces of film (F1, F2, F3, F4 and F5) samples were prepared by diluting 1 mL of SP extract with 10 mL of mobile phase (dilution 1:10 v/v).

I.2.3.6. Quantitative determination of potassium sorbate by HPLC

The quantitative determination of SP was conducted through HPLC according to Pylypiw & Grether (2000) and Buonocore et al. (2003), with minor modifications. The HPLC operating mode was isocratic, the injection volume was 20 µL and the column was at ambient temperature. The chromatography column was a Grace C-18 Reverse phase, 250×4.6 mm, 5 µm (Grace, Columbia, MD, USA). Sample data collection was optimized to 20 min per sample with UV detection at 255 nm. The optimal flow rate was determined to be 0.8 mL/min, according to Pylypiw & Grether (2000). The concentration of SP in the pieces of film evaluated was calculated by 7 point external standard method. The method had a detection limit of 0.1 mg/L.

I.2.4. Statistical analysis

Results are reported as the mean of measurements. To determine whether significant differences ($p < 0.05$) existed among the mean values, the one-way analysis of variance (ANOVA) and Duncan's multiple range test were performed using STATISTICA 8.0 for Windows (StatSoft Inc., Tulsa, OK, USA).

I.3. RESULTS AND DISCUSSION

I.3.1. The basic characterization of composite pellets

The functional characteristics of OV and OVA films, with the latter obtained by adding SP micropowder into polymeric composite matrix during the extrusion process, were essentially related to the process conditions and to the properties of the raw materials involved. The raw thermoplastic HDPE/$CaCO_3$/TiO_2 material was supplied as dried pellets, hermetically sealed into plastic bags. Once the bag was open, pellets looked white in colour as shown in Fig. 12.

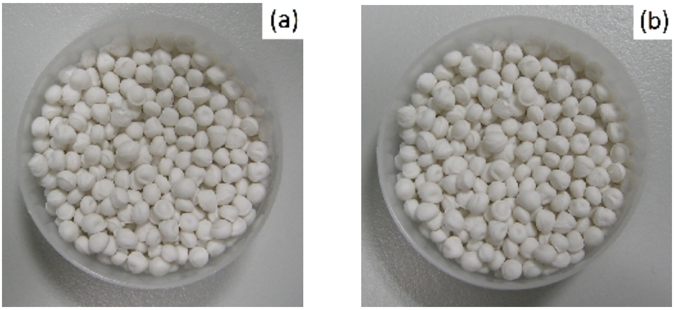


Figure 12. *Pictures of pellets as supplied (a) and after 6 months of air exposition (20°C, 45-50% R.H.).*

Comparing the pellets as supplied with the sample exposed at air for 6 months, it was evident that no significant differences were presented in terms of colour. Also the colour measurements performed by mean of a colorimeter had confirmed this behaviour (Tab. 4). Thus, it is reasonable to think that the air exposition has not influences on the white appearance of pellets.

Table 4. *Chroma index measured on pellets as supplied and after 6 months of air exposition (20°C, 45-50% R.H.).*

	L^*	a^*	b^*
Pellets as supplied	96.5 ± 0.6	+5.1 ± 0.1	-2.2 ± 0.5
Pellets after 6 months of air exposition	96.4 ± 0.6	+5.0 ± 0.2	-2.2 ± 0.4

Data are mean ± standard deviation

This white aspect was voluntarily obtained through the addition of $CaCO_3$ and TiO_2 in micropowder form. Generally, these harmless inorganic minerals are added for their whiteness effect but it is well known their use in polyolefin fillers in order to improve plastic drawbacks such as low barrier properties or low stiffness (Avella et al., 2007). For example, $CaCO_3$ is considered an abundant filler widely used both for cost reduction and for beneficial effects on mechanical properties such as an increase in modulus and impact strength (Almeras et al., 2003), while TiO_2 is one of the most efficient white pigment for its high refractive index and, at the same time, its absorption of UV light can confer durability to materials (Allen et al., 2009).
In order to assess how the HDPE matrix was filled, a total ash content evaluation was performed. This test showed a pellet ash content of 33 ± 0.5%, which represented a measure of total inorganic elements, such as Ca and Ti, in the pellets. This finding was confirmed also through the morphological characterization of HDPE/$CaCO_3$/TiO_2 pellet surfaces. ESEM micrographs rose in evidence that pellet displayed slightly rough surfaces, mainly due to the presence of particles gathered in small agglomerations irregular in shape and with sizes of several micrometers, as shown in Fig. 13. At the same time, EDS analysis pointed out a homogeneous distribution of different elements, such as C, Ca, O and Ti, on pellet surfaces.

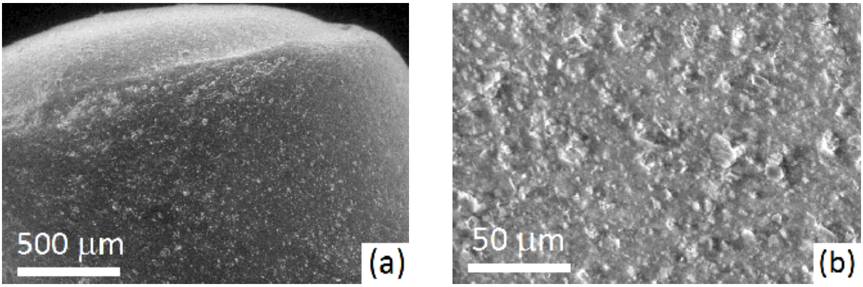


Figure 13. *ESEM micrographs of pellet surfaces at 100× (a) and 1000× (b) magnification factor.*

This superficial exposition of inorganic elements has inevitably influenced some chemical-physics characteristics of the pellets, such as the pH value. Regarding that the aqueous extract of pellets presented a pH value of 9.9 ± 0.1, it is evident that the composite monolayer film obtained by blown film extrusion process will show similar properties.

I.3.2. The influence of formulation on films characteristics

The basic characterization of pellets pointed out the homogeneous superficial presence of inorganic compounds that could influence the final properties of the films produced. In order to understand the effect of the inorganic minerals and the subsequent active substance addition into the HDPE matrix in terms of feasibility to realize food packaging films, monolayer OV and OVA films were produced by an industrial extrusion process. A single screw extruder was preferred because it exerts shear levels able to guarantee a reasonable dispersion but not as high to promote increases of friction, temperature and finally the material degradation (van Zuilichem et al., 1999). Considering their low moisture content, ranged from 0.01 to 0.03% (w/w), pellets were not dried before the extrusion. Thus, the selected conditions for the process allowed the films to be achieved as thin layer which displayed gas permeability values according with the polyolephinic nature of the HDPE matrix and better mechanical properties than pure HDPE (≥ 99% w/w purity) (Tab. 2, p. 15). After the winding, films were worked to obtain sheets. Finally, the thermoplastic films were flexible and extendable with a homogeneous white opaque tone (Fig. 14), depending on pellet formulation.

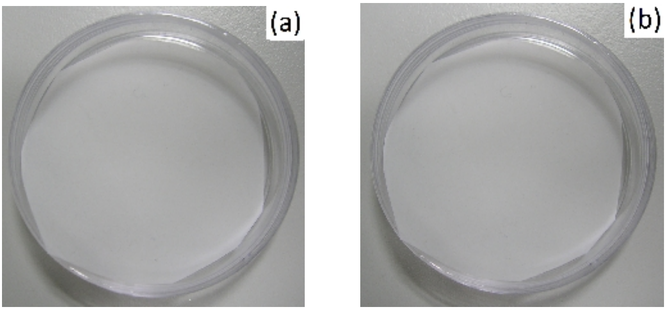


Figure 14. *Pictures of OV film as realized (a) and after 6 months of air exposition (20°C, 45-50% RH).*

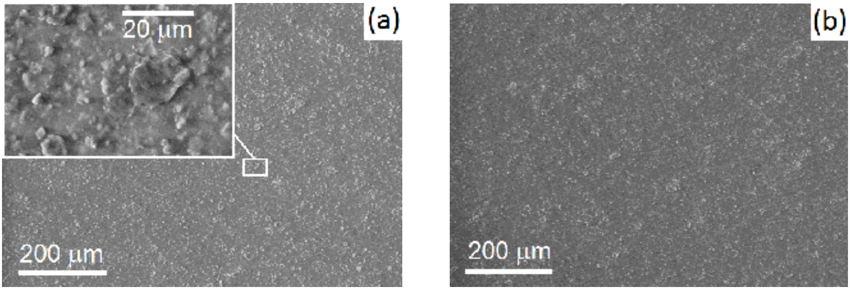
Also for the films, comparing the superficial appearance in terms of colour, there were no significant differences between the samples as realized and exposed at air for 6 months. This finding was confirmed by colour measurements (Tab. 5). The white opaque surfaces showed by films have not only an importance for marketing or appealing aspects, but it has a fundamental role to reduce light-induced reactions kinetics. It is generally considered that light influences some chemical reactions that lead to food spoilage. In particular, light-induced reactions include the destruction of chlorophyll in vegetables, the discoloration of fresh meats, the destruction of riboflavin in milk, the photosensitized oxidation of vitamin C and carotenoid pigments and so on (Huis in't Veld, 1996; Min & Zhang, 2005). Above these assumptions, it is evident that the use of a food packaging able to reduce the food exposure to light is one of the most effective means of preventing light-induced chemical spoilage (Piergiovanni & Limbo, 2010a).

Table 5. *Chroma index measured on films as realized and after 6 months of air exposition (20°C, 45-50% R.H.).*

	L^*	a^*	b^*
OV film as realized	97.3 ± 0.2	+5.4 ± 0.1	-3.0 ± 0.3
OV film after 6 months of air exposition	97.3 ± 0.4	+5.3 ± 0.2	-3.0 ± 0.2
OVA film as realized	97.2 ± 0.3	+5.4 ± 0.2	-2.9 ± 0.2
OVA film after 6 months of air exposition	97.1 ± 0.4	+5.4 ± 0.3	-3.0 ± 0.2

Data are mean ± standard deviation

As we have already emphasized, films surfaces presented a white opaque tone depending on pellet formulation. ESEM micrographs recorded for OV and OVA films have confirmed the latter assumption. In fact, both the inner and outer sides of each sample films displayed slightly rough surfaces, mainly due to the presence of inorganic particles which were gathered in small agglomerations irregular in shape and with sizes of several micrometers (Fig. 15). Furthermore, comparing Figures 15a, 15b and 15c, it is evident that film surfaces do not present any deformations defects. A defective surface, showed in Fig. 15c, is due to different issues during extrusion process, such as high temperature, high rotating screw speed, low degassing capacity or presence of impurities, that exerting excessive shear levels into the extruder barrel (van Zuilichem et al., 1999).



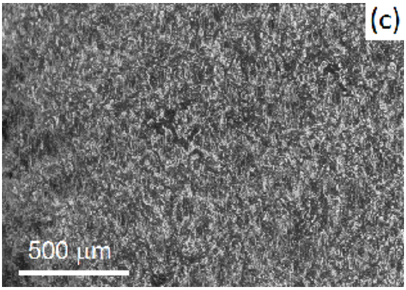


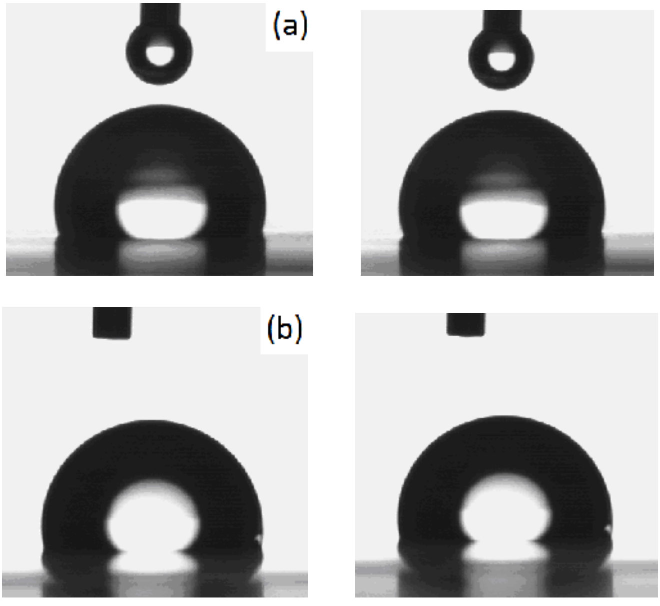
Figure 15. *ESEM micrographs of outer (a), inner (b) and defective (c) OV film surfaces at 200× and 1600× (a), 200× (b) and 100× (c) magnification factor.*

In our case, the use of a single screw extruder permitted to obtain a reasonable dispersion of HDPE matrix and inorganic particles. This assertion was confirmed by EDS analysis performed on films surfaces. It has pointed out the homogeneous distribution of different elements, such as C, Ca, O and Ti, on OV surfaces while OVA surfaces displayed also the presence of K. These findings agreed with the formulation of the thermoplastic composite material where the inorganic minerals were added with the aim to improve whiteness and tactile effect. Considering their high refractive index, it is clear that the analyzed films are able to reduce the food exposure to light.

However, the superficial exposition of inorganic particles has given particular properties to the produced films. For example, the aqueous extract of each films sample showed pH values of 10.2 ± 0.2. These results are dependent to the high solubility of $CaCO_3$ in pure water (Kralj & Brečević, 1995), but they also suggest that similar amounts of superficial $CaCO_3$ can migrate from films to an aqueous solution. This migration underlines that OV and OVA films present the same chemical-physical behaviour independently from the addition of SP powder during the extrusion process. Nevertheless $CaCO_3$ is included into the Union Positive List and approved for use in food contact plastic, films have to be monitored in order to satisfy the migration limit defined by the Council Regulation (EC) No 10/2011 (Commission of the European Communities, 2011). Considering that the solubility of $CaCO_3$ depends strongly on pH and that, generally, the decrease in pH increases the $CaCO_3$ solubility (Meller, 1984), it is evident that OV and OVA films will be not suitable for contact with acidic foodstuffs (at pH values lower than 4.5 as defined by Council Regulation (EC) No 10/2011).

I.3.3. The surface hydrophobicity as an evaluation parameter

Further to the significant chemical-physical influence, inorganic particles added to the HDPE matrix are able to affect the surface properties of films. In our work, changes in film hydrophobicity were studied by surface characterization. Surface properties were investigated for a better understanding of the inorganic particles role in final films, comparing OV and OVA films with other food packaging films, such as HDPE and PP. Hydrophobicity of all samples were determined by measuring water contact angle of the films surface used for food contact. Considering the machine direction during the manufacture steps of slitting and reeling, OV and OVA surfaces used for food contact were them included in the inner side of the bubble. The sessile drop method gives good indication of material surface properties with a comparative study at time 0 and time 60 s (Karbowiak et al., 2006b). When the pure water drop was deposited onto the film surface (time 0), the initial water contact angle was evaluated. Then, a second measurement was performed on the same drop shape after 60 s, corresponding to a metastable equilibrium between the liquid droplet and the tested surface. Fig. 16 shows the images of water drop shapes, acquired at time 0 and 60 s, of HDPE, PP, OV and OVA surfaces used for food contact.



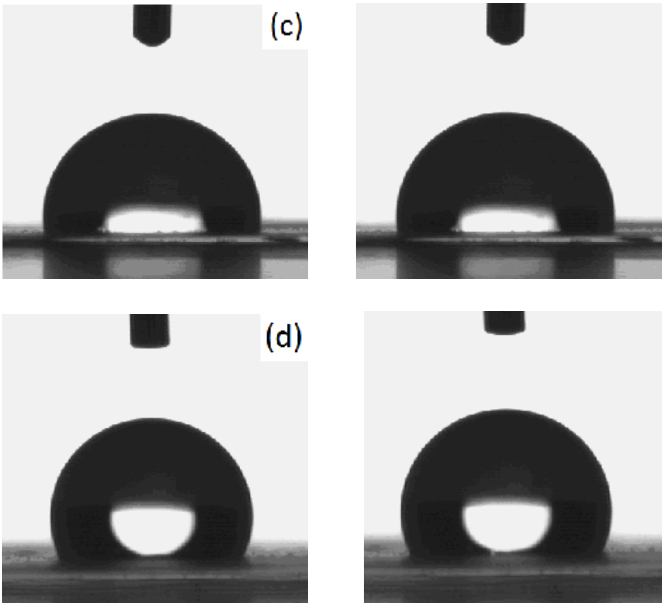


Figure 16. *Water drop shapes, acquired at time 0 and time 60 s, of HDPE (a), PP (b), OV (c) and OVA (d) films for contact angle measurement.*

As we can see, there is only a slight decrease of the water drop shape between 0 and 60 s for both film evaluated. This behaviour was essentially due to evaporation phenomenon because synthetic polymers have generally hydrophobic surfaces characterized by high contact angles with water. These materials are indeed non-absorbent and then phenomena such as absorption, solvation or swelling were negligible (Karbowiak et al., 2006b; Owens & Wendt, 1969; Schneider, 1996). These assumptions according to the results collected and showed in Fig. 17. All films presented hydrophobic properties characterized by high water contact angle around 90-115°. While the surface properties of HDPE and PP films were similar, according to Owens & Wendt (1969) and Schneider (1996), OV and OVA showed significant differences in terms of hydrophobicity. Observing OV film contact angles, it is evident that the superficial exposition of inorganic particles, displayed by ESEM-EDS analysis, influenced not only the chemical-physical behaviour of the film, but also its surface properties. The well known hydrophilic character of $CaCO_3$ (Premphet & Horanont, 2000), together with its large amount in the composite formulation, may influence the wettability of film that results less hydrophobic than pure synthetics polymers.

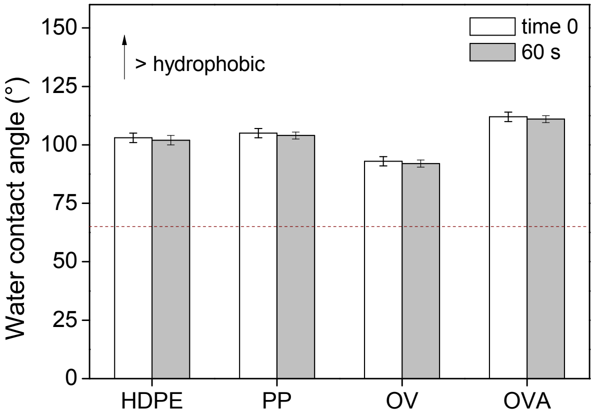


Figure 17. *Water contact angle acquired at time 0 and 60 s, of HDPE, PP, OV and OVA films.*

However, the addition of SP during the extrusion process seems to promote higher hydrophobic properties. The significant increase in hydrophobicity on OVA film surface suggests a higher availability of hydrophobic groups probably due to a particular dispersion of formulation components during the production process. In this phase, the blend between two different raw components, such as SP micropowder and composite pellets, may involve different interactions between polymeric matrix, inorganic particles and sorbate agent, inducing a higher exposure of hydrophobic groups in final OVA film surface.

As observed, surface properties are conditioned by the formulation of the composite film and their interactions. However, even if the inorganic particles amount was high, the presence of a synthetic matrix, such as HDPE, enhances the hydrophobic characteristics of final film surface.

I.3.4. Evaluation of preservative presence and distribution in OVA film

The analyses showed previously revealed that OV film can be considered as hydrophobic continuous media with a homogeneous structure. Basing on these assumptions, we tried to use this basic film as a carrier of preservatives in order to improve its functionality and give it new functions. The resultant OVA film, obtained by adding SP micropowder into polymeric composite matrix during the extrusion process, presented similar properties displaying a higher surface hydrophobicity.

During this last ten years many Authors have studied and developed active packaging systems adding SP, through coating or drying applications, onto different matrix (Choi et al., 2005; Jipa et al., 2012; Sayanjali et al., 2011; Uz & Altinkaya, 2011). Contrarily, SP incorporation through extrusion process was achieved by a few Authors (Flores et al., 2010; Han, 2000). The aim of antimicrobial food packaging is to transfer the antimicrobial agent from the film carrier to the food surface, concentrating them at the food surface where the protection is needed (Buonocore et al., 2003). Many Authors (Buonocore et al., 2003; Choi et al., 2005; Graciano-Verdugo et al., 2010; Granda-Restrepo et al., 2009; Guillard et al., 2009; Han, 2000) have investigated the potentiality of their proposed antimicrobial food packaging describing the release kinetics of the active agent to relate its migration to the antimicrobial efficacy of the coating produced in lab scale.

In our case, considering all complexities of an industrial extrusion process in terms of temperature, raw quantities and process management, we will evaluate essentially the surface distribution of the active agent. To reach this goal, we analyzed 5 different samples of a random area of the produced film. Then, considering that SP is soluble in water and its solubility increases with temperature (Sofos & Busta, 1981), we performed an ultrasound (UT) assisted extraction of sorbate agent using water as extracting solvent.

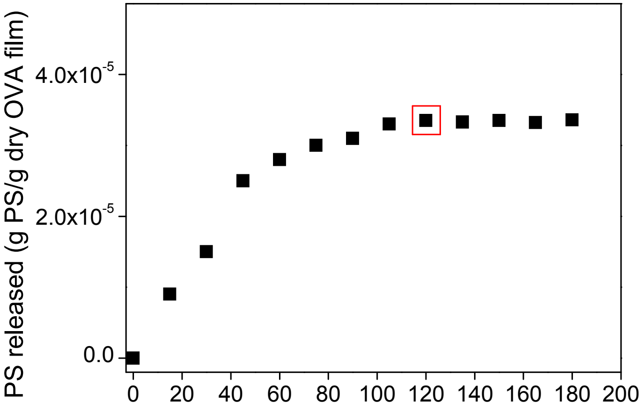


Figure 18. *Amount of SP released plotted as a function of ultrasound assisted extraction time.*

The UT assisted extraction was optimized by monitoring, by means of an HPLC, the SP concentration. A constant amount of preservative agent released into water was reached after 2 hours of UT application, as shown in Fig. 18. The five water extracts were investigated using a HPLC analysis according to Pylypiw & Grether (2000) and Buonocore et al. (2003), with minor modifications. The sorbate concentration was calculated by 7 point external standard method, as presented in Fig. 19.

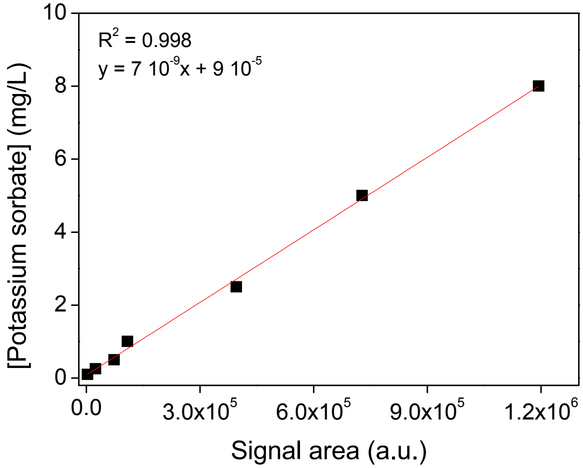


Figure 19. *Calibration of potassium sorbate.*

Theoretically, each water extract can have 30 mg/L of SP (corresponding to 0.3 mg of SP into each investigated film with a composite material mass of 43 mg) in case of total active agent extraction. Considering the hypothesis that a part of SP was chemically bonded to the polymer backbone and the dilution (1:10 v/v) of each extract sample, we carried out the SP calibration from 0.1 to 8 mg/L. The plot (Fig. 19) shows a linear response according to the following equation: [SP] (mg/L) = $7 \cdot 10^{-9}$·signal area (a.u.) + $9 \cdot 10^{-5}$ with $R^2 = 0.998$.

The HPLC assay showed the sole presence of sorbate into all sample extracts, recorded with an elution time of 13 min, as presented in Fig. 20. However, SP distribution was scarcely homogeneous and the recovering data ranged from 5 to 22%, where % w/w was expressed as fraction of the initial amount of SP blended with pellets. All results are resumed in Tab. 6.

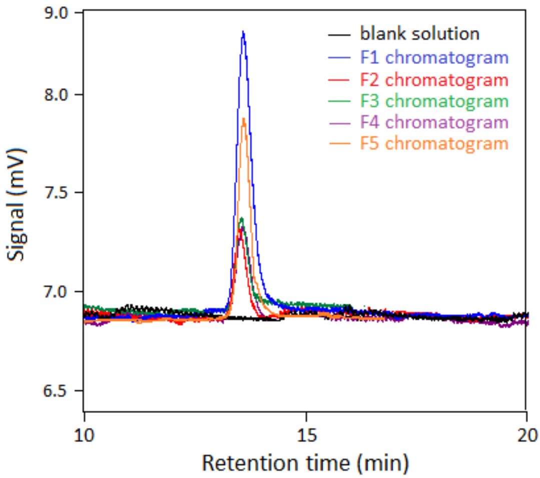


Figure 20. *HPLC chromatograms of blank solution and F1-F5 samples extracts. Flow rate: 0.8 mL/min; injecting volume: 20 µL.*

Table 6. *Potassium sorbate concentration for each water extract expressed in mg/mL and % w/w. Values followed by different letter are significantly different at the p-level of 5 %.*

Sample	[SP] in water extract sample (mg/mL)	SP in sample* (%)
F1	6.7 ± 0.8^{a}	22.3
F2	1.4 ± 0.1^{d}	4.7
F3	1.8 ± 0.2^{c}	6.0
F4	1.6 ± 0.2^{cd}	5.3
F5	4.0 ± 0.4^{b}	13.3

* ([ ] extract·100)/[ ] theoretical
Data are mean ± standard deviation

These evidences show that only a small amount of the initial added SP keep available at the OVA film surface. This loss together with the verified sorbate inhomogeneous distribution may be ascribed to loose occurring during extrusion process, to its irreversible bonding to the polymer matrix, according to Buonocore et al. (2003), and/or to its partition coefficient between film and simulant. Above these findings, it seems that ESEM-EDS results are in disagreement with the latter. The morphological characterization performed using ESEM-EDS is generally considered an analysis which involves over a small specimen area, in our case small strips of film (Danilatos, 1988). Probably, analyzed strips presented a homogeneous elemental distribution of K in the small area investigated by EDS. However using a different analysis that involves a higher sample area, the same OVA film exhibited the effective presence of SP, but it resulted inhomogeneously distributed on the surface.

I.4. CONCLUSIONS

- The raw thermoplastic HDPE/$CaCO_3$/TiO_2 pellets looked a stable white colour voluntarily obtained through the addition of inorganic particles, such as $CaCO_3$ and TiO_2, into the polymeric matrix.

- Composite dried pellets showed a high ash content due to the large amount of total inorganic elements in the pellets. These particles were homogeneously distributed in the pellet surfaces and gathered in small agglomerations irregular in shape and with sizes of several micrometers.

- This superficial exposition of inorganic elements influenced chemical-physics characteristics of the pellets. For example, the aqueous extract of pellets presented an alkaline pH.

- Thermoplastic OV and OVA films, produced through blown film extrusion, were flexible and extendable with a homogeneous and stable white opaque tone depending by the homogeneous distribution of inorganic elements on films surfaces. The superficial exposition of particles with high refractive index was able to reduce the food exposure to light.

- The use of a single screw extruder permitted to obtain a reasonable dispersion of HDPE matrix and inorganic particles.

- Also the aqueous extract of films showed similar alkaline pH. These values were dependent to the high solubility of $CaCO_3$ in pure water. Its migration into water at ambient temperature suggests that films OV and OVA films will be not suitable for contact with acidic foodstuffs.

- OV and OVA film presented non-absorbent surfaces used for food contact with hydrophobic properties similar to other films for food packaging based on pure synthetic polymers, such as HDPE and PP.

- OV and OVA films showed significant differences in terms of hydrophobicity because the addition of SP during the extrusion process may promote higher hydrophobic properties.

- OV film seems to be a good carrier of preservatives, in fact the resultant OVA film presented available SP on the film surface. However SP distribution was scarcely homogeneous and the recovering data ranged from 5 to 22% of the initial amount of SP blended with pellets during extrusion process.

PART II

The role of TiO_2: a photocatalytic implication

II. GENERAL SECTION

The OV thermoplastic film previously characterized was produced using a composite pellet made by two main components such as a synthetic matrix (HDPE) and an inorganic mineral with whiteness properties ($CaCO_3$) used to reduce the content of the plastic polymeric amount. However, also a small amount of rutile TiO_2 was embedded in film to improve whiteness and brightness.

Considering the well known photocatalytic activity of TiO_2 (Fujishima & Honda, 1972; Mills & Le Hunte, 1997; Srinivasan & Somasundaram, 2003; Teh & Mohamed, 2011), in this section we presented the results of an investigation on the TiO_2 photocatalytic activity displayed by OV film. With this purpose, its photocatalytic properties were compared with those displayed by aqueous suspensions of commercial TiO_2 micro- and nano-powders. This investigation was carried out using voltammetric, spectrophotometric, gas chromatographic and water contact angle measurements.

II.1.1. The role of titanium dioxide (TiO_2)

II.1.1.1. Basic characteristics of TiO_2

TiO_2, also known as titanium (IV) oxide or titania, is the naturally occurring oxide of titanium. It is a pale yellowish or white colour solid which exists in three crystalline forms known as anatase, rutile and brookite (Fig. 21). In each crystalline form, the Ti ion coordinates with six O atoms, which in turn are linked to three Ti atoms, and so on (Cromer & Herrington, 1955; Watanabe et al., 1999). In particular (Srinivasan & Somasundaram, 2003):

- rutile phase has a primitive tetragonal unit cell and it is the most common TiO_2 form;
- anatase phase has the same rutile primitive unit cell, but there is no relation between the interfacial angles of the two minerals, except in the prism-zone of 45° and 90°;
- brookite phase has a orthorhombic unit cell and it is not economically significant since there is no abundant supply in nature.

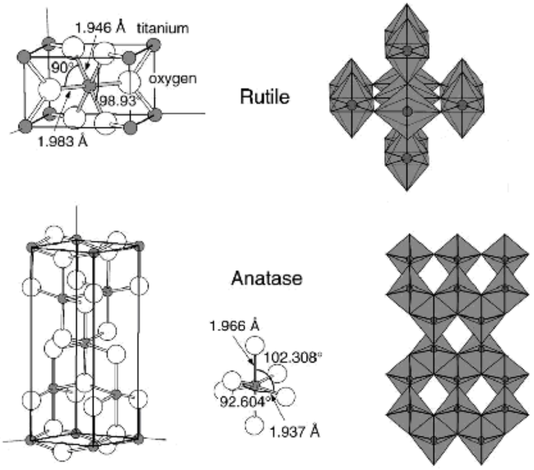


Figure 21. *Bulk structures of rutile and anatase. In both structures, slightly distorted octahedral are the basic building units. The bond lengths and angles of the octahedrally coordinated Ti atoms are indicated and the stacking of the octahedral in both structures is shown on the right side.*

Generally, TiO_2 is considered an inert, nontoxic and inexpensive mineral, whose high refractive index and high capability to absorb UV light make it an interesting white pigment and environmental friendly catalyst (Allen et al., 2002; Fujishima & Honda, 1972). The ability of TiO_2 to reflect and refract light more efficiently than any other pigment is due to the internal crystal structure of pure oxide, which permits to reflect all the wavelength of the visible light to the same degree, producing the effect of whiteness to the human eye. All these attributes, together with its opacity, are achieved for a particle diameter around 0.2-0.4 µm (Allen et al., 2005; Zhang et al., 2005). However, crystal structure and size are reported as factors affecting also TiO_2 photocatalytic activity (Saravanan et al., 2009). The use of TiO_2 as photocatalyst in several fields has received greater attention since the discovery of photocatalytic cleavage of water on TiO_2 electrodes by Fujishima & Honda (1972).

II.1.1.2. Applications of TiO_2

TiO_2 is widely employed as a white pigment into products such as paints, coatings, plastics, papers, inks, foods (i.e. used to whiten skinned milk), medicines and cosmetics. In these cases, TiO_2 particles are submicroscopic with size distributions narrower than monodisperse particulates. However, considering its well known photocatalytic activity, TiO_2 is also used in environmental and purification treatment (Bougheloum & Messalhi, 2009; Cho et al., 2004; Choi et al., 2007; Hussain et al., 2011; Wang et al., 2010). In particular, a growing interest is paid to its use in highly dispersed form (as micro- and nano-particles) on inert materials, so as to avoid problems such as particles agglomeration, catalyst recovery and loss of irradiation efficiency (Li et al., 2011; Zhang et al., 2009). Several supports have been adopted, such as ceramic (Wu et al., 2007), glass (Behnajady et al., 2008; Watanabe et al., 1999), activated carbon (Li Puma et al., 2008; Zhang et al., 2010) and zeolite (Ko et al., 2009), with the aim to promote multiple functions performed by TiO_2, such as decomposition of pullutants (Bougheloum & Messalhi, 2009; Choi et al., 2007; Gaya & Abdullah, 2008) and inactivation of pathogenic microorganisms (Chawengkijwanich & Hayata, 2008; Cho et al., 2004; Maneerat & Hayata, 2006; Maness et al., 1999).

Since photocatalytic reactions mainly take place on the surface of the catalyst, a high surface-to-volume ratio is of great significance for increasing these reactions (Zhonghou et al., 2008). Thus, TiO_2 incorporation into thin films based on polymers may be conceivably an effective approach to photoactive filmable materials. A number of methods have been reported for the preparation of TiO_2 thin films, including chemical vapour deposition (Zhang et al., 2006), flame spraying (Kavitha et al., 2007), spray pyrolysis (Li et al., 2005; Rogers et al., 2004), laser ablation (Liu et al., 2009) and reactive evaporation (Fan et al., 2006). This notwithstanding, until now the direct addition of TiO_2 powders in food packaging films during an extrusion process has not been investigated and only coating, deposition or spraying applications have been proposed (Chawengkijwanich & Hayata, 2008; Kubacka et al., 2007; Maneerat & Hayata, 2008; Zhou et al., 2009).

II.1.1.3. Mechanism of photocatalytic reaction

From the perspective of material science, TiO_2 is considered a semiconductor usually characterized by an electronic band structure in which the lowest empty energy bands, called the conduction band (CB), and the highest occupied energy band, called the valence band (VB), are separated by a band-gap (E_{bg}). TiO_2 can use the near UV radiation (λ: 350-387 nm) for photocatalysis because it has this suitable energetic separation (Saravanan et al., 2009). In fact, when photons with energy equal or higher than E_{bg} reach the surface of the particle, TiO_2 photoexcitation occurs. TiO_2

electrons are promoted from the filled VB to the empty CB simultaneously with the generation of positive holes in VB (Eq. 1).

$$TiO_2 + h\nu \rightarrow TiO_2\,(e^- + h^+) \qquad (1)$$

Then, e^- and h^+ pairs migrate to the surface of catalyst where redox reactions can occur involving both h^+ for oxidation and e^- for reduction processes, respectively. In particular, holes are capable of oxidizing an organic substrate (RX) adsorbed on the surface or break apart water molecules to form the hydrogen cation (H^+) and the hydroxyl anion (OH^-), which will then lead to the production of strong oxidizing $^{\bullet}OH$ radicals (Eqs 2-4) (Mills & LeHunte, 1997).

$$TiO_2\,(h^+) + RX_{ad} \rightarrow TiO_2 + {}^{\bullet}RX_{ad}^{\;+} \qquad (2)$$
$$TiO_2\,(h^+) + H_2O_{ad} \rightarrow TiO_2 + H^+ + OH^-_{ad} \qquad (3)$$
$$TiO_2\,(h^+) + OH^-_{ad} \rightarrow TiO_2 + {}^{\bullet}OH_{ad} \qquad (4)$$

Meanwhile, the electrons promoted to CB can reduce the oxygen molecule to superoxide anion ($O^{\bullet}{}_2^-$) (Eq. 5).

$$TiO_2\,(e^-) + O_2 \rightarrow TiO_2 + O^{\bullet}{}_2^- \qquad (5)$$

These reactive radicals can produce other reactive species, such as perhydroxy radical ($^{\bullet}OOH$) and H_2O_2 (Eqs 6-8), and/or decompose organic compounds (Fujishima et al., 2000).

$${}^{\bullet}O_2^- + H^+ \rightarrow {}^{\bullet}OOH \qquad (6)$$
$${}^{\bullet}O_2^- + {}^{\bullet}OOH + H^+ \rightarrow O_2 + H_2O_2 \qquad (7)$$
$$TiO_2\,(e^-) + H_2O_2 \rightarrow TiO_2 + OH^- + {}^{\bullet}OH \qquad (8)$$

Photoexcitation and electron-transfer reactions on a TiO_2 particle are resumed in Fig. 22.

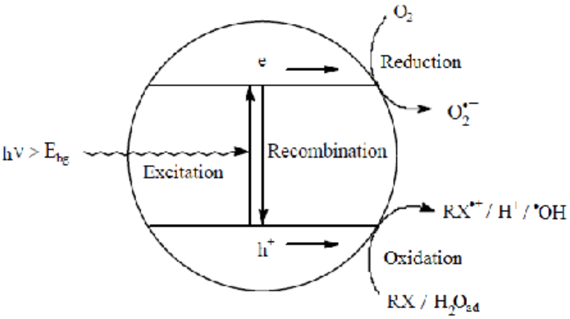


Figure 22. *Photoexcitation and electron-transfer reactions on a TiO_2 particle (Srinivasan & Somasundaram, 2003).*

The overall catalytic performance of TiO_2 particles mainly depends on its intrinsic properties, such as crystal structure and specific surface area (Litter, 1999). In particular, large surface area exhibited by small particle size is favourable to improve photocatalytic activity (Alvaro et al., 2006). Furthermore, it is well known that the photocatalytic activity of the anatase phase is better than the rutile phase because anatase phase has a higher E_{bg} than rutile phase (3.2 eV for anatase; 3.03 eV for rutile) (Schiavello, 1997). Somarjai (1986) has also showed that the highest activity of anatase phase can be due to the recombination of the electron-hole pair that occurs more rapidly on the surface of the rutile phase. As a consequence, the amounts of reactants and hydroxides attached to the surface of the rutile phase are smaller than those of the anatase phase.

II.1.1.4. The photoinduced superhydrophilicity

As reported in literature, the photoexcitation of TiO_2 lead to photocatalysis processes which are responsible of both breakdown of organic compounds and surface superhydrophilicity (Wang et al., 1997). The photoinduced superhydrophilicity was firstly discovered by accident in work that was being carried out at the laboratories of TOTO Inc., in 1995. It was found that a TiO_2 film acquires superhydrophilic properties after UV illumination (Fujishima et al., 2000).

This discovery has prompted extensive research on TiO_2 characteristics in order to better understanding the superhydrophilicity phenomenon. It is widely accepted that the atomic coordinations at the TiO_2 surface differ from those in the bulk since the atom arrangements are truncated on the surface. This gives rise to coordinated oxygen atoms, which are protruding from the crystal surface and more energetically reactive than the usual titanium present in the bulk of anatase and rutile crystals (Watanabe et al., 1999). When TiO_2 is UV irradiated, electron-hole pairs are generated and oxygen vacancies are created at the two-coordinated bridging sites because electrons tend to reduce the Ti^{4+} cations to the Ti^{3+} state and the hole reacts with the bridging sites, causing their oxidation (Highfield & Grätzel, 1988) as reported in Fig. 23. Water molecules can heal these oxygen defects through their coordination at the Ti^{3+} sites. Consequently, the coordinated water molecules release a proton for charge compensation and then a new OH group forms, leading to the increase in the number of OH groups which tend to make the surface hydrophilic (Henderson, 1996). Based on the above assumptions, it seems that hydrophilic conversion depends on the bridging oxygen sites presence.

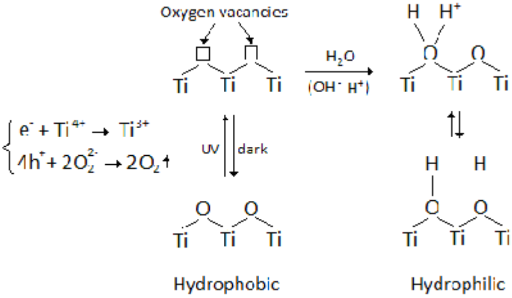


Figure 23. *Schematic illustration of photoinduced superhydrophilicity mechanism.*

Nevertheless, many Authors (Wang et al., 1999; Watanabe et al., 1999) have demonstrated that the wettability conversion is not dependent only to the photoinduction but also to the TiO_2 crystal faces because they have not the same number of bridging oxygens protruded.

II.1.2. Evaluation of TiO_2 photocatalytic activity

Semiconductors with a wide energy band gap, such as TiO_2, have been widely studied for their photoinduced surface activation responsible of both decomposition of organic compounds and induction of surface hydrophylicity. This investigation focus on the evaluation of photocatalytic activity of TiO_2 embedded in OV films obtained by blown extrusion. This activity was estimated in comparison to that displayed by aqueous suspensions of commercial TiO_2 micro- and nano-powders by either voltammetric, UV-vis spectrophotometric measurements.

II.1.2.1. Electrochemical approach

Considering the mechanism of photocatalysis discussed above, our interest was to evaluate the photoactivity of TiO_2 embedded into OV films monitoring the photogeneration of high reactive radicals, such as $^{\bullet}OH$. This goal was reached exploiting an electroanalytical approach originally proposed by Wang et al. (2009) to test the antioxidant activity of natural extracts, in which 4-hydroxybenzoic acid (4-HBA) was used as electroactive $^{\bullet}OH$ scavanger.

II.1.2.1.1. Basics of voltammetry

Linear sweep voltammetry (LSV) and cyclic voltammetry (CV) are the most widely used voltammetric techniques for studying redox reactions of both organic and inorganic compounds. These electroanalytical methods provide informations not only on the electrochemical quantities typical of a redox process, but also on possible chemical reactions coupled with charge transfer steps. In these electroanalytical techniques the electrode can be used as a tool for producing reactive species in a small solution layer surrounding its surface and at the same time for monitoring chemical reactions in which they are involved. Moreover, as the relevant responses can be obtained within a few milliseconds after stimulation of the electrode, they may be used for studying even very fast reactions, thus allowing detection of short-lived transient intermediates. In both LSV and CV, a small ($< 0.1\ cm^2$) stationary working electrode is dipped in an unstirred solution containing an excess of supporting electrolyte to repress migration of charged reactants and products, so that any transfer of electroactive species to and from the electrode surface can occur only through diffusion. In LSV the potential of the working electrode changes linearly with time starting from a potential E_i where no electrode reaction occurs and moving to the potential where reduction (more negative values) or oxidation (more positive values) of the investigated analyte takes place; the instantaneous potential E_t applied at time t is given by Eq. 9:

$$E_t = E_i \pm vt \qquad (9)$$

where v is the scan (or sweep) rate, that is, the absolute value of the rate of the potential change, dE/dt, which is constant within any sweep. The sign depends on the potential scan direction (positive for anodic sweeps and negative for cathodic sweeps). The current is measured throughout the experiment and the resulting current-potential curve (voltammogram) displays the typical shape shown in the lower plot in Fig. 24a, which also reports the main parameters. They are peak current (i_p), that is, the maximum current value; peak potential (E_p), that is, the potential corresponding to i_p; and half-peak potential ($E_{p/2}$), that is, the potential at which $i = i_{p/2}$.

Peak position on the potential scale (E_p) is related to the formal potential of the redox process and to its reversibility degree (conditioning also the peak shape $E_p - E_{p/2}$), thus providing information on the analyte involved. Instead, peak height (i_p) gives information on the analyte concentration, the number of electrons involved in the electrochemical process, and the possible presence of coupled chemical reactions. The voltage waveform applied to the working electrode in CV is shown in the upper plot of Fig. 24b.

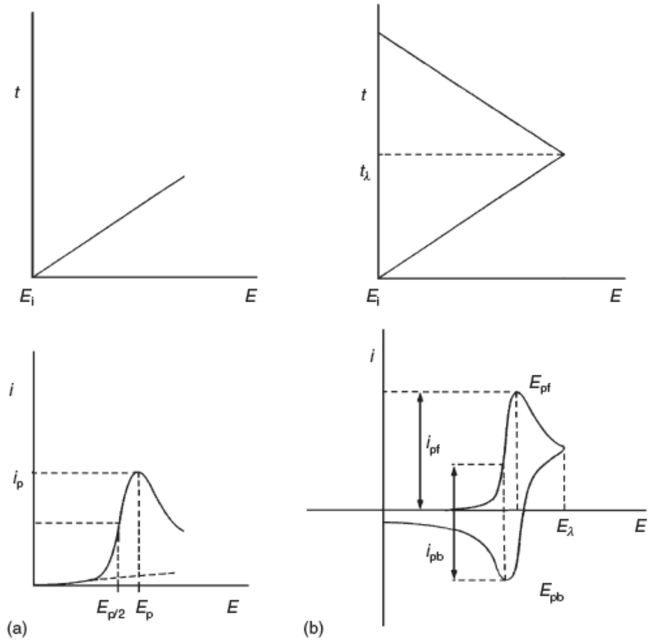


Figure 24. *Potential waveforms for linear sweep (a) and cyclic (b) voltammetry and the resulting voltammograms.*

Until t_λ the electrode potential is varied linearly with time as in LSV, while at t_λ the potential scan direction is reversed, so that the waveform becomes the combination of two joined linear sweeps. The switching potential E_λ is in fact both the final potential for the forward scan and the initial potential for the reverse sweep. As a result, the potential E_t applied to the working electrode in the forward scan is once again described by Eq. 9, whereas E_t for the backward scan (t_λ<t<2t_λ) is given by Eq. 10:

$$E_t = E_i \pm vt \pm v(t - t_\lambda) = E_i \pm 2vt_\lambda \pm vt \qquad (10)$$

This equation applies to symmetrical triangular waveforms, which are the most commonly adopted in CV, even though other waveforms have also been proposed (Bontempelli & Toniolo, 2009).

II.1.2.1.2. Differential pulse voltammetry (DPV) measurements

Pulse voltammetric techniques permit convienient quantitation down to the 10^{-8} M concentration level increasing the ratio between the faradaic and nonfaradaic currents. The various pulse techniques are all based on a sampled current potential-step experiment. A sequence of such potential steps, each with duration of about 50 ms, is applied to the working electrode. After the potential is stepped, the charging currents decays rapidly (exponentially) to a negligible value, while the faradaic current decays more slowly. Thus, by sampling the current late in the pulse life, an effective discrimination against the charging current is achieved (Wang, 2001).

The difference between the various pulse voltammetric techniques is the excitation waveform and the current sampling regime. In differential pulse voltammetry, fixed-magnitude pulses superimposed on a linear potential ramp are applied to the working electrode, as shown in Fig. 25.

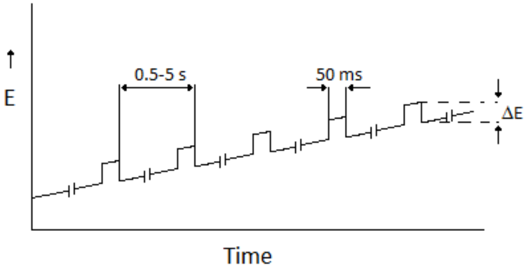


Figure 25. *Excitation signal for differential pulse voltammetry.*

The current is sampled twice, just before the pulse application and at the end of the pulse time. The first current is instrumentally subtracted from the second and this current difference is the value plotted versus the applied potential. The resulting differential pulse voltammogram consists of current peaks, the height of which is directly proportional to the concentration of the corresponding analytes (Galus, 1994).

II.1.2.2. Spectrophotometric approach

To evaluate the photocatalytic activity of TiO_2 particles, UV-Vis spectrophotometric tests were performed by many Authors (Böttcher et al., 2010; Wang et al., 2010; Wu & Zhang, 2004). Different dyestuffs were used as organic compounds and their decomposition was monitored by determining the absorbance through a UV-Vis spectrophotometer. This approach was chosen because spectrophotometry permits to measure the absorbance properties of a solution as a function of wavelength. In particular, Wu & Zhang (2004) and Wang et al. (2010) proposed the use of rhodamine B solution to evaluate the photocatalytic activity of pure TiO_2 sheets, while Böttcher et al. (2010) tested different dyestuffs, such as methylene blue, rhodamine B and other azo dyes, monitoring the decrease of dyes solutions absorbance. As they have demonstrated, dyes solutions absorbance falls towards zero, revealing the organic compounds degradation under UV-Vis irradiation.

II.1.2.3. Water contact angle measurements

To evaluate the photocatalytic activity of TiO_2 based materials, photoinduced hydrophilicity monitored by many Authors exploiting water contact angle measurements (Miyauchi et al., 2002; Wang et al., 1997; Wang et al., 1999; Watanabe et al., 1999). In particular this approach has been applied to test the photoinduced hydrophilicity on TiO_2 coated glass and TiO_2 thin films surfaces. As a result, they have showed the slower hydrophilic conversion upon UV irradiation and faster hydrophobic conversion when the surfaces were maintained in the dark (Wang et al., 1998; Watanabe et al., 1999). These surface properties were revealed both to the decrease of surfaces water contact angle towards small values around 0-10° upon UV irradiation and to the increase of these values when the samples were seated in the dark.

II. EXPERIMENTAL SECTION

II.2. MATERIALS AND METHODS

Voltammetric, spectrophotometric and gas chromatographic measurements here presented were carried out in the laboratories of the Department of Chemical Sciences (University of Udine), under the supervision of Prof. Toniolo.

II.2.1. Materials, reagents and solutions

Commercial 25 nm (99.7% w/w metal basis) and 1 μm (99.9% w/w metal basis) TiO_2 anatase powders, purchased from Aldrich (Sigma-Aldrich, Seelze, Germany), were used as reference materials to compare with the photocatalytic activity of OV composite ($HDPE/CaCO_3/TiO_2$ $HDPE/CaCO_3/TiO_2$ in the w/w ratio of 49:50:1%, respectively) film, where TiO_2 was in the form of rutile micropowder (0.2-0.3 μm). 4-hydroxybenzoic acid (4-HBA) and 3,4-dihydroxybenzoic acid (3,4-DHBA), purchased from Aldrich (Sigma-Aldrich, Seelze, Germany), were employed as hydroxyl radical scavengers probes. Methylene blue (MB), obtained from Fluka (Fluka AG, Buchs, Switzerland), was employed as daylight sensitive probe. Sodium dihydrogen phosphate monohydrate was purchased from Fluka (Fluka AG, Buchs, Switzerland) and used to prepare phosphate buffer solutions. High purity deionized water ($R > 18 M\Omega cm^{-1}$, $0.055\ \mu S cm^{-1}$, TOC < 5 ppb) was obtained with a Milli-Q Integral system (Millipore Co., Billerica, MA, USA). All gases employed (He, CH_4, N_2 and CO_2) were of ultrapure grade (SIAD S.p.A., Bergamo, Italy).

II.2.2. Electrochemical approach

II.2.2.1. Apparatus

Differential pulse voltammetry (DPV) measurements were performed in conventional 50 mL undivided three-electrode cells and carried out with a potentiostat Autolab PGSTAT 30 (Metrohm Autolab B.V., Utrecht, The Netherlands) driven by a dedicated software installed on a Pentium IV computer. The counter electrode was a platinum sheet, while an $Ag/AgCl,Cl^-_{sat}$ electrode was used as the reference electrode. The static working electrode was a glassy carbon disk, with an apparent geometric area of 0.07 cm^2, which was mirror-polished with graded alumina powder prior to each set of experiments. Irradiation was carried out employing a 15 W UV lamp with a spectral maximum wavelength of 365 nm (Fischer Scientific SAS, Illkirch, France) 10 cm high over the reaction mixture.

II.2.2.2. Operating procedure

The 4-HBA decay was monitored by DPV measurements carried out in an undivided three electrode cells containing 20 mL of a 2 mM 4-HBA solution in 0.1 M phosphate buffer (pH 7.1) in which either 100 µg mL^{-1} of each TiO_2 reference powder or 64 cm^2 of OV film sample (containing the same amount of TiO_2) were suspended or immersed. OV film was brought in contact with the reaction mixture only on the side used for food contact (inner side of blown film produced). Stirred reaction mixtures were then irradiated with a wavelength band centered at 365 nm for 8 h and periodically analysed by DPV at defined times. Blank DPV measurements were also carried out with time on the same solution in the absence of TiO_2. For each defined time, three measurements were performed at ambient temperature.

II.2.3. Spectrophotometric and gas chromatographic approaches

II.2.3.1. Apparatuses

UV-Vis spectrophotometric measurements were conducted using a spectrophotometer GENESYS 10 (Thermo Electron Co., Waltham, MA, USA). HS-GC measurements were carried out by a GC-TCD 5890 series II (Hewlett Packard, Palo Alto, CA, USA) equipped with a Carboxen 1006 (30 m × 0,53 mm, average thickness 30 µm) column (SUPELCO, Bellefonte, PA, USA). Irradiation was performed employing a 100 W incandescent lamp (OSRAM AG, Munich, Germany) with the aim to simulate daylight irradiation.

II.2.3.2. Operating procedure

The decay of MB was monitored on 20 mL of a 0.04 mM MB deaerated aqueous solution to which either 100 µg mL^{-1} of each TiO_2 reference powder or 64 cm^2 of film sample were added. These samples were introduced into a suitable 100 mL gas tight vessel and continuously stirred and irradiated with the daylight lamp placed at about 10 cm over the solution. OV film was brought in contact with the MB deaerated aqueous solution only on the film side used for food contact. Small volumes (0.5

mL) of both the reaction solution and its headspace were periodically sampled and analysed by monitoring spectrophotometrically the absorbance at 660 nm and by detecting the CO_2 content by GC-TCD, respectively.

II.2.4. Evaluation of photoinduced hydrophilicity

The surface superhydrophilicity as increase of film wettability during a light irradiation time were evaluated from contact angle measurements using a goniometer (Kruss GmbH, Hamburg, Germany) equipped with an image analysis software (Drop Shape Analysis, Kruss GmbH, Hamburg, Germany). A droplet (3 µL) of high purity deionized water ($R > 18 M\Omega cm^{-1}$, $0.055\ \mu S cm^{-1}$, TOC < 5 ppb) was deposited with a precision syringe on the film surface used for food contact. The sessile drop method was used and the water contact angle θ was obtained as function of time (0 and 60 s). The θ of OV film and pure HDPE film, kindly delivered by Poliflex (Poliflex S.r.l., Brescia, Italy), were periodically measured during 8 h of daylight irradiation performed by means of a 100 W incandescent lamp (OSRAM AG, Munich, Germany) placed at about 10 cm over the surface of analyzed films. Ten measurements per film surface were carried out. All films were pre-conditioned in a chamber under the same environmental conditions to avoid interferences due to competing moisture exchange at the surface around the droplet.

Water contact measurements were carried out in the laboratories of the Department of Food Science (University of Udine).

II.2.5. Statistical analysis

Results are reported as the mean of measurements. To determine whether significant differences ($p < 0.05$) existed among the mean values, the one-way analysis of variance (ANOVA) and Duncan's multiple range test were performed using STATISTICA 8.0 for Windows (StatSoft Inc., Tulsa, OK, USA).

II.3. RESULTS AND DISCUSSION

II.3.1. The electrochemical behaviour of 4-HBA and 3,4-DHBA

It is well known that TiO_2 undergoes absorption in the near UV region and is consequently able to promote photocatalytic reactions when the energy input is equal or higher than its band-gap energy (Litter, 1999; Srinivasan & Somasundaram, 2003). When this condition is achieved, mobile electrons and holes are generated on the TiO_2 surface. These holes can act as powerful oxidizing centres toward adsorbed organic molecules, either directly or indirectly by the mediation of hydroxyl radicals ($^{\bullet}OH$) produced by oxidation of adsorbed water molecules (Mills & LeHunte, 1997). The electrochemical approach here adopted to evaluate the TiO_2 photocatalytic activity was based on the use of 4-HBA as an effective trapper of $^{\bullet}OH$, according to Wang et al. (2009). The choice of 4-HBA was made in view of its intrinsic redox activity (oxidation occurs at +0.8 V vs Ag/AgCl Cl^-_{sat}) and its ability to react with $^{\bullet}OH$ to form only 3,4-DHBA as an isomer, whose oxidation occurs at a potential value (+0.3-0.4 V) lower than that required by the parent 4-HBA (Hu et al., 2008; Ste-Marie et al., 1996). DPV measurements performed on blank solution, 2 mM 4-HBA and 0.1 mM 3,4-DHBA are presented in Fig. 26.

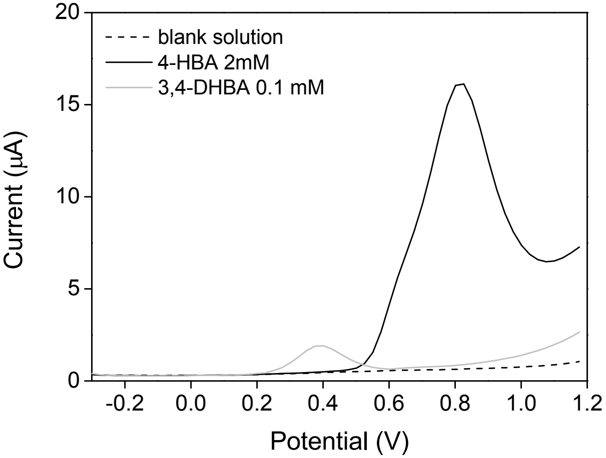


Figure 26. *DPV profiles recorded in 0.1 M phosphate buffer (pH 7.1) without hydroxyl radical scavengers probes and with 2 mM 4-HBA or 0.1 mM 3,4-DHBA. DPV conditions: modulation time 0.075 s; step potential 25 mV; modulation amplitude 50 mV; scan rate 50* $mV\ s^{-1}$.

As displayed in Fig. 26, 4-HBA and 3,4-DHBA showed different electrochemical characteristics, according to Hu et al. (2008) and Ste-Marie et al. (1996). Then the calibrations of 4-HBA and 3,4-DHBA were executed and the DPV results collected are presented in Fig. 27.

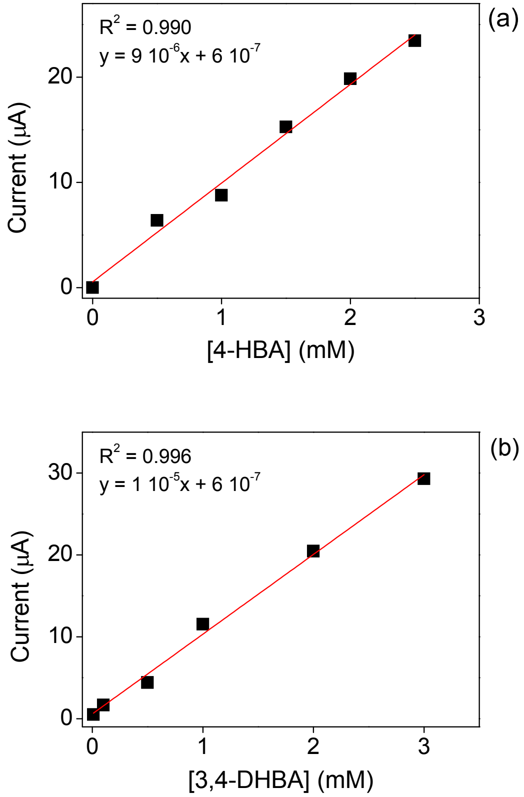
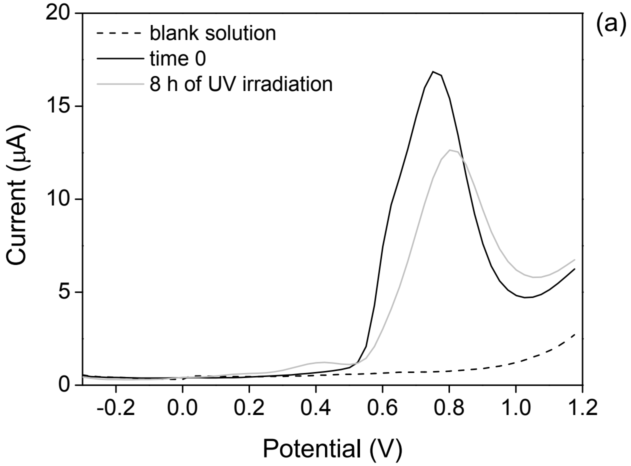


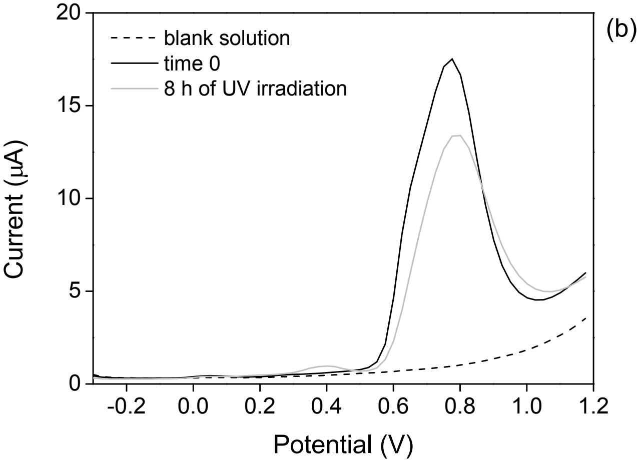
Figure 27. *Calibration of 4-HBA (a) and 3,4-DHBA (b).*

Calibration of 4-HBA was carried out from 0.01 mM to 2.5 mM showing a linear response according to the following equation I (A) = $9 \cdot 10^{-6} \cdot C_{4\text{-HBA}}$ (mM) + $6 \cdot 10^{-7}$ with $R^2 = 0.990$, while calibration of 3,4-DHBA was carried out from 0.01 mM to 3 mM showing a linear response according to the following equation I (A) = $1 \cdot 10^{-5} \cdot C_{3,4\text{-DHBA}}$ (mM) + $6 \cdot 10^{-7}$ with $R^2 = 0.996$. These calibrations allowed us to quantify the concentrations of 4-HBA and 3,4-DHBA when produced.

II.3.2. The photocatalytic activity evaluated by DPV

When 4-HBA was used as $^{\bullet}OH$ scavenger, its decay was followed by carrying out periodically DPV scans. Fig. 28 compares typical DPVs recorded after UV irradiation on a 25 nm and 1 µm anatase TiO_2 suspensions with those found under the same experimental conditions when the anatase powder was replaced by a OV film sample containing an equal amount of TiO_2, but in this case in rutile crystalline form.





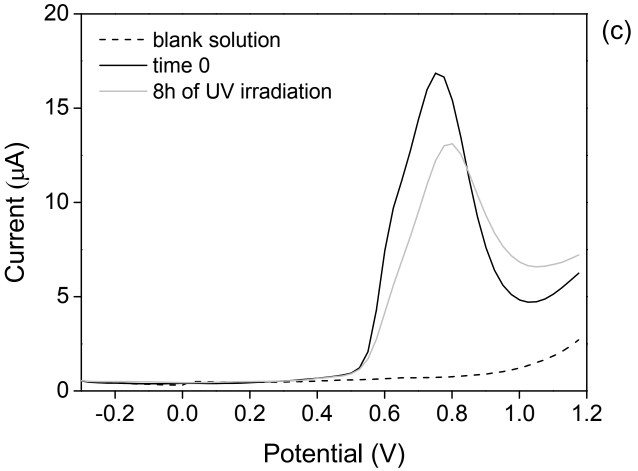


Figure 28. *DPV profiles recorded in 2 mM 4-HBA solutions in 0.1 M phosphate buffer (pH 7.1) in which 25 nm (a) and 1 µm (b) anatase TiO$_2$ (100 µg mL^{-1}) or 64 cm^2 of OV film (c) were suspended or immersed, respectively. DPV conditions: modulation time 0.075 s; step potential 25 mV; modulation amplitude 50 mV; scan rate 50 mV s^{-1}.*

These voltammograms showed a comparable decrease of 4-HBA oxidation peak for both types of samples, whereas the appearance of a peak current at about +0.3-0.4 V due to 3,4-DHBA oxidation was observed for 25 nm and 1 µm anatase samples, even though with a height quite smaller than the height decrease recorded for 4-HBA.

DPV results obtained by exposing samples to UV irradiation for different time are summarized in Fig. 29, where the decay with the irradiation time of 4-HBA (Fig. 29a) is compared with the progressive increase of 3,4-DHBA (Fig. 29b). These figures points out that the 4-HBA decay caused by the photocatalytic activity of TiO$_2$ embedded in the film is comparable to that promoted by anatase TiO$_2$ reference powders, the only difference being the 3,4-DHBA formation which is observed only for the reference samples even though its concentration seem to attain a steady state value. Furthermore, observing Fig. 29a and 29b, it is possible to infer that all samples exhibit a quite similar photodegradation activity towards 4-HBA. At the same time, the formation of 3,4-DHBA result clearly crystal form- and size-dependent, according to many Authors (Alvaro et al., 2006; Litter, 1999; Somorjai, 1986).

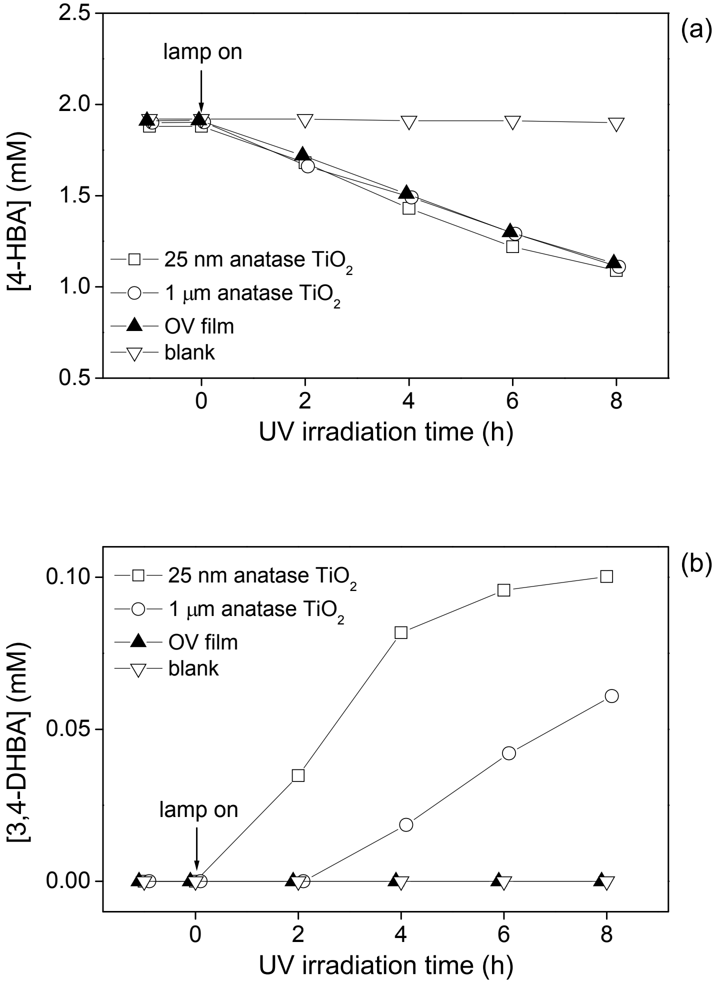


Figure 29. *4-HBA decay (a) and 3,4-DHBA formation (b) detected with the irradiation time by DPV measurements for the samples indicated. All points are the mean of three tests repeated under the same condition. The relative standard deviation was always ≤ 3.0 %.*

The absence of signals relative to the 3,4-DHBA production during OV film sample irradiation can be conceivably ascribed to the fact that TiO_2 holes available on the film surface are able to promote the further oxidation of this product too. In fact, by suspending a OV film in a 0.1 M phosphate buffer solution (pH 7.1) to which 3,4-DHBA was added, a rapid decay of this species after UV irradiation was observed.

II.3.3. The photocatalytic activity evaluated by spectrophotometry and HS-GC

To obtain more insight about the photodegradation activity displayed by OV film, additional spectrophotometric and HS-GC measurements were performed on MB solution used as the reference photodegradation molecular probe. Also in this case, the photocatalytic activity showed by composite OV film resulted totally comparable with that displayed by anatase TiO_2 samples, as reported in Fig. 30.

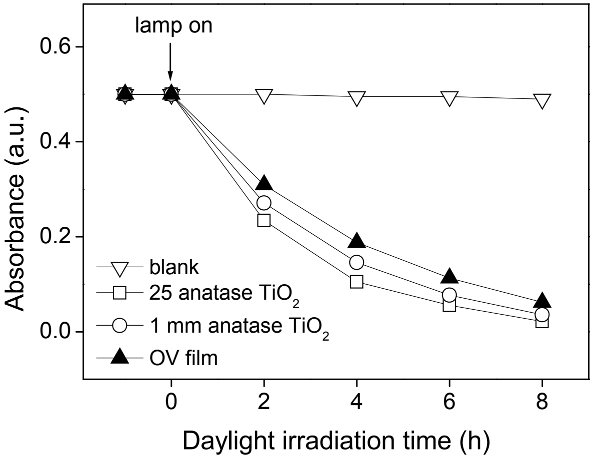


Figure 30. *MB decay detected with the irradiation time by spectrophotometric measurements. All points are the mean of three tests repeated under the same condition. The relative standard deviation was always ≤ 3.0 %.*

Fig. 31 put in evidence the decolouration of MB solution under irradiation.

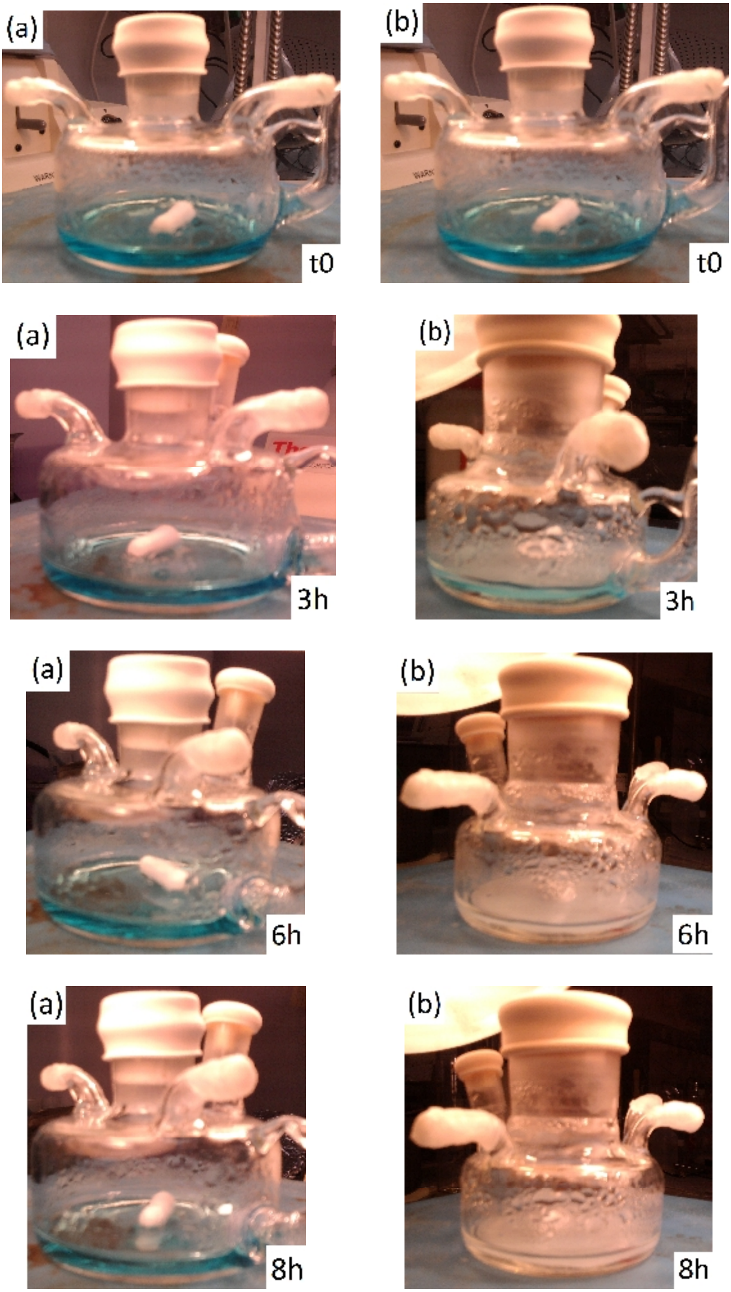


Figure 31. *Colour changes of MB solutions without suspended sample (a) and with OV film immersed (b) during daylight irradiation.*

At the same time, a satisfactory overlap of the CO_2 formed concomitantly as the final product is also observed, as shown in Fig. 32.

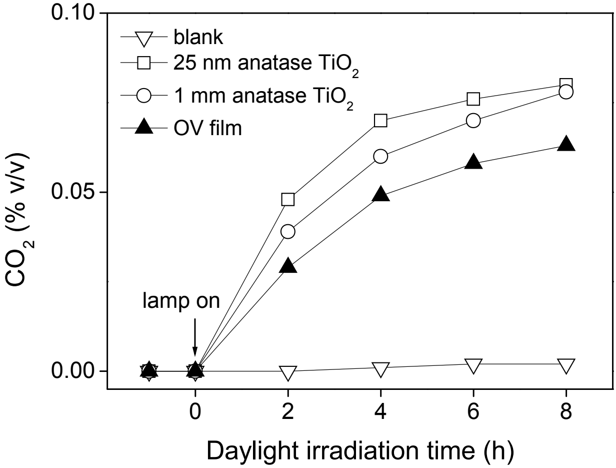


Figure 32. *CO_2 formation detected with the irradiation time by GC-TCD measurements. All points are the mean of three tests repeated under the same condition. The relative standard deviation was always ≤ 3.0 %.*

In particular, the headspace analysis monitored on OV film sample pointed out CO_2 production, whose maximum concentration was estimated to be ca. 0.06% v/v. The lower CO_2 yield compared to that expected (0.08% v/v) from a quantitative conversion of the MB into CO_2 may be related to an uncompleted oxidation process leading to leuco-species. The production of CO_2 derived from the dye photodegradation assisted by TiO_2 was observed and discussed by many Authors (Ma & Yao, 1998; Machado et al., 2000; Penpolcharoen et al., 2001; Wu et al., 1998). In particular, Ma & Yao (1998) and Wu et al. (1998) concluded that, under UV-Vis irradiation, the photodegradation of dye solutions undergo two possible pathways, such as photocatalytic and photosensitization. On the whole, our work pointed out a clear photodegradation of organic compounds promoted by OV film through an oxidation process leading to CO_2 production.

II.3.4. The evaluation of OV film photoinduced hydrophilicity

To confirm the photocatalytic activity of the OV film its photoinduced hydrophilicity was also investigated. These tests, carried out on the film surface used for food contact, were performed measuring the water contact angle and comparing the obtained values with those displayed by a pure HDPE film. Fig. 33 shows the images of water drop shapes, acquired respectively at 0 and 60 s after water drop deposition on the OV film.

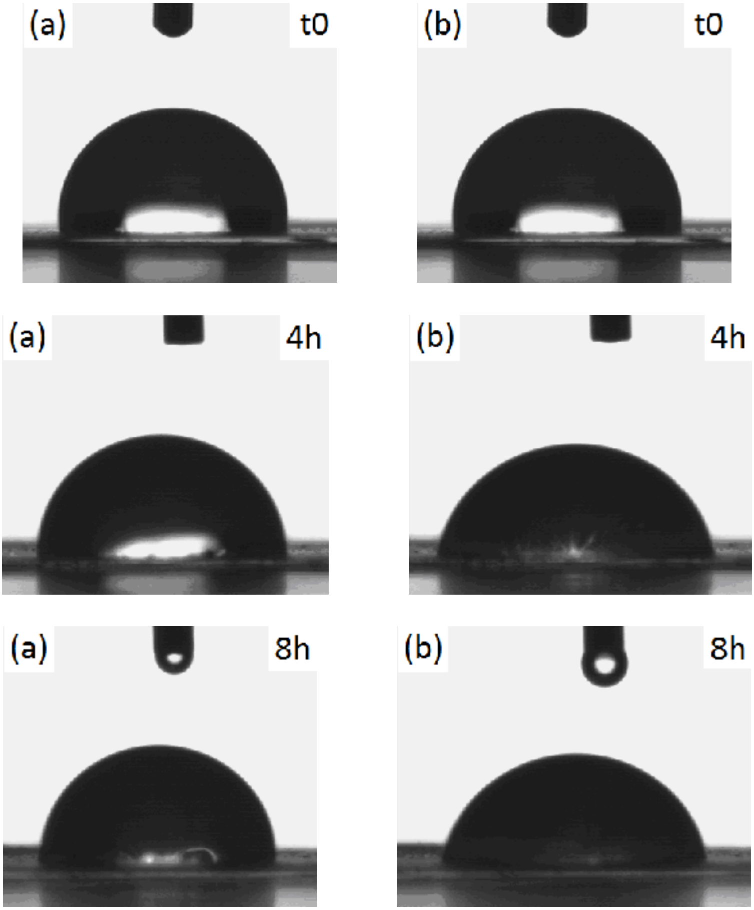


Figure 33. *Water drop shapes on OV film acquired respectively at 0 (a) and 60 s (b) after water drop deposition. Images were collected periodically during the daylight exposure.*

As can be observed there is a slight decrease of the water drop shape with the daylight irradiation time. Above all, these tests show a decrease of the drop shape between 0 and 60 s which became higher increasing the irradiation time. This behaviour may be ascribed to a slight water absorption by the studied OV film in agreement with that observed by Karbowiak et al. (2006b) on edible films. This effect tends to be more pronounced increasing the time of daylight exposure which seems able to influence the affinity between water and surface (Wang et al., 1999).

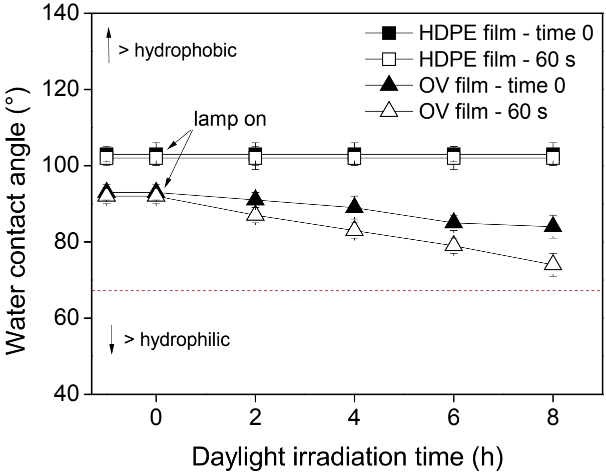


Figure 34. *Water contact angle at time 0 and 60 s of HDPE and OV films acquired periodically during the daylight exposure.*

Fig. 34 shows that the presence of inorganic particles into the composite formulation of OV film can influence its surface properties. In particular, the HDPE film displayed a non-absorbent surface with higher water contact angles than OV film surface. No modifications seem to occur on a HDPE film during daylight exposure and only a slight decrease of the water drop shape between 0 and 60 s was measured, essentially due to the evaporation phenomenon. On the contrary, the daylight exposure is able to influence OV surface properties during the irradiation time making it less hydrophobic.

II.4. CONCLUSIONS

- This study demonstrated that the OV film here investigated was characterized by a profitable and reproducible photocatalytic activity.

- DPV measurements performed on 4-HBA under UV irradiation pointed out that OV film had a photocatalytic activity totally comparable with that displayed by anatase TiO_2 micro- and nano-powder samples. The absence of signals relative to the 3,4-DHBA production during OV film sample irradiation can be ascribed to the fact that TiO_2 holes available on the film surface are able to promote the further oxidation of this product too.

- Spectrophotometric and gas chromatographic measurements performed on MB as reference photodegradation molecular probe confirmed that OV film was able to promote the oxidation of organic compounds with production of CO_2 as the final degradation product. This behaviour was totally comparable with that displayed by anatase TiO_2 micro- and nano-powder samples.

- Water contact angle measurements showed a significative water contact angle decrease with daylight irradiation time for OV film demonstrating a clear light induced surface affinity towards water. However, this light induced hydrophilic conversion was lower than that reported in literature for pure TiO_2 thin film. This different behaviour can be ascribed to the composite nature of OV film.

PART III

Evaluation of film effects on real foodstuffs preservation

III. GENERAL SECTION

Evidences collected in the previous chapters have showed that the OV composite film was a flexible material characterized by homogeneous structure, hydrophobic surface and photocatalytic activity. To improve its functionality, OV film was used as a carrier of preservatives. Potassium sorbate was chosen as active agent exploiting both its well known ability to inhibit the growth of numerous microorganisms, such as yeasts, molds and selective bacteria, and its stability at high temperature ($T_m \sim 270°C$) (Sofos & Busta, 1981). The resultant OVA film presented similar properties displaying higher surface hydrophobicity than OV film. However, also if sorbate was keep available on the film surface, its distribution was scarcely homogeneous. This defect may influence the OVA film effectiveness. For these reasons, our objective in this third chapter was to evaluate the effects of food packaging on the appearance and mechanical and rheological properties of two real traditional food matrices sensitive to microbiological spoilage, such as mozzarella and stracchino cheeses.

III.1.1. Secondary conversion of flexible films for food packaging

III.1.1.1. From monolayer to multilayer film

OV and OVA films are characterized by high flexibility and extensibility. Therefore, they may find large applications in the food packaging field as wrapping materials. These practices are chosen when the packaged food is in solid state, because the latter does not require particular mechanical protection. However, for foodstuffs in other physical state, such as dairy products, yoghurt, confectionery products etc., the use of rigid and semi-rigid thermoformed containers are preferred. These forms of packaging may be shaped to the specifics of packaged food and are able to improve higher barrier properties (Piergiovanni & Limbo, 2010b). Considering their basic characteristics, monolayer films made by plastic can be combined with other plastics using different processes, such as coextrusion, lamination and coating, to achieve properties which the components could not provide alone (Kirwan & Strawbridge, 2003).

III.1.1.2. The main characteristics of a lamination process

The plastic lamination is a production process exploited to combine two or more films using adhesives (wet lamination) or heat (dry lamination).

Generally, the lamination is an alternative to the coextrusion process where two or more layers of molten plastic are combined during film manufacture. The choice of the process depends on several factors. In particular, the process of coextrusion can produce both wastes which are not recyclable and multilayer films that can only be surface printed (Defosse, 1999). On the contrary, the laminated multilayer films may be reverse printed, but they can suffer from curling when pushed or pulled. Thus, the choice about the approach to take must be assessed on case by case, considering both technical and commercial factors (Piergiovanni & Limbo, 2010b).

A wide range of adhesives is available to perform the combination between different plastic films in wet lamination. However, there are many adhesives that can adhere satisfactorily to certain plastic matrix, but they have not good moisture barrier, while other can perform excellent barrier properties together with higher drying period required before to use the laminate. For example, polyvinyl acetate (PVA) and other water-based adhesives may not adhere satisfactorily to polyolefines, such as PE and PP, while polyurethanes and other cross-linking adhesives are preferred for their barrier properties. Adhesives are normally applied from a gravure roller, keeping combined films under pressure. Then, the solvents are removed using heating or UV radiation. In some film structures, the adhesive may react with the film coatings and produce discolouration or migration phenomena. To avoid these problems, it is possible to join different monolayer films by their passing through a heated nip roller system, as shown in Fig. 35 (Kirwan & Strawbridge, 2003).

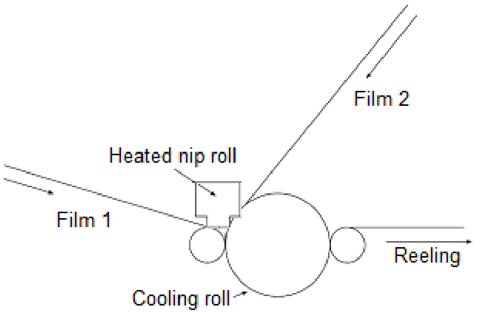


Figure 35. *A schematic representation of a dry lamination process (Kirwan & Strawbridge, 2003).*

Dry lamination process relies on the similar sealing point of the films involved (Tab. 1, p. 13). In fact, films approached to the T_m passing under the heated roll; as a consequence, their adhesion can be achieved through the pressure performed by cooling rolls and then a new formed laminate was obtained. PS, ethylene-vinyl acetate (EVA) and ethylene-vinyl alcohol (EVOH) is the most used middle layer of laminates based on polyolefines, such as PE, PP and PET. In particular, EVOH is preferred in order to improve barrier properties of the final laminate film.
After the production process, the laminate is reeled and then thermoformed into different shapes, such as food trays and so on (Piergiovanni & Limbo, 2010b).

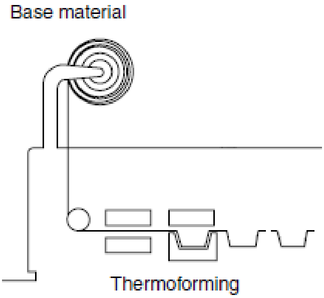


Figure 36. *A schematic representation of a thermoforming process (Kirwan & Strawbridge, 2003).*

III.1.2. Mozzarella cheese as real food matrix

III.1.2.1. Mozzarella cheese: specifics and manufacture

Mozzarella is an Italian traditional *pasta filata* type cheese, characterized by a typical ball shape and a brilliant white colour. The term is used for several kinds of Italian cheeses, such as buffalo, *fior di latte*, low moisture and smoked mozzarella, which differ by milk type and production process (Ribero et al., 2007). Generally, the manufacture of this cheese begins with the addition of a mesophilic or thermophilic starter into the milk batch. The starter has the role to produce enough lactic acid during cheese making to demineralise and transform the curd into a state that will stretch in hot water at the target pH (Sinigaglia et al., 2008; Zisu & Shah, 2007). In fact, after the coagulation performed by the addition of the calf rennet, the curd is cut to large pieces, stirred, heated and placed in a hoop to form a solid mass. At pH lower than 5.5 the curd mass can be kneaded and stretched in hot water until a smooth, fibrous structure is obtained (Ribero et al., 2007; Zisu & Shah, 2007). The resultant mozzarella cheese is packaged traditionally in a dilute solution of salts (NaCl and/or $CaCl_2$), called conditioning brine. In the USA, the addition of a small amount of TiO_2 (< 1% w/v) in conditioning brine is permitted. This practice is performed with the aim to intensify the surface whiteness of mozzarella cheese. Even though TiO_2 is listed among the admitted food colorants and classified with the E number 171 in the Council Regulation (EC) No 1333/2008 (Commission of the European Communities, 2008), the Italian Ministerial Decree of 27 February 2008 establishes that the use of TiO_2 in conditioning brine and cheese formulation is forbidden. Moreover, also the use of other food additives is admitted conditionally in the formulation of an Italian mozzarella cheese. For example, the Italian law (Italian Ministerial Decree No 209 of 27 February 1996) establishes that the use of citric acid, acetic acid, lactic acid, glucono delta-lactone and cellulose is admitted, while the potassium sorbate (E 202) is forbidden. Thus, the shelf life of the packaged Italian mozzarella cheese is assessed around 18-24 days and then its basic characteristics are lost (Conte et al., 2007; Kuo & Gunaderakan, 2003).

III.1.2.2. Mozzarella cheese characteristics

Composition and structural characteristics affect cheese mechanical properties and they undergo notable changes during storage with the proteolysis that being the principal driving force behind age-dependent functional changes in Mozzarella cheese (Kindstedt, 2004; Subramanian & Gunasekaran, 1997). The majority of these characteristics are associated both with the surface colour and the mechanical properties of the mozzarella cheese (Sheehan et al., 2005). As a consequence, many Authors have been studied the appearance and the mechanical properties of mozzarella cheese to investigate the effects on the cheese shelf life of different treatments performed during the production process.

Tunick et al. (1995) compared two different mozzarella cheeses derived from different homogenized skim milk to understand the homogenization role in the definition of mechanical properties. These Authors used the texture profile analysis (TPA) to determine hardness and springiness of cheese.

Sheehan et al. (2005) investigated the effects of high pressure treatment in order to produce a mozzarella cheese with reduced-fat content. Authors have performed a TPA to obtain some information about mechanical

properties and a determination of colour by means of a colorimeter to assess the appearance changes during the storage.
Also Van Hekken et al. (2007) performed a TPA to evaluate the changes in texture parameters (hardness, springiness, gumminess and cohesiveness) with the increase of storage time. Finally, Bermúdez-Aguirre & Barbosa-Cánovas (2011) evaluated the effects of ω-3 incorporation on the appearance and the mechanical properties of mozzarella cheese using colour measurements and a TPA. The latter was used to evaluate physical characteristics such as hardness, cohesiveness, springiness, chewiness, gumminess and resilience.

III.1.3. Stracchino cheese as real food matrix

III.1.3.1. Stracchino cheese: specifics and manufacture

Stracchino is an Italian traditional soft cheese, made from pasteurized cow milk to which starter culture and calf rennet are added to obtain a mixed coagulation. The originated curd is then cut in slices and left to ripen at 30°C for several days. The final product has a flat shape with a thin skin and a white or mildly yellowish paste. Generally, stracchino cheese is packaged in modified atmosphere and it has 18-21 days of refrigerated storage life (Stecchini et al., 1996).

III.1.3.2. Experimental review

To our knowledge, only few studies have been conducted on stracchino cheese and their characteristics (Bramanti et al., 2003; Bramanti et al., 2004; Stecchini et al., 1996). Stracchino cheese is a food matrix that for its viscoelastic characteristics may be studied using fundamental dynamic tests (Belitz et al., 2009).

III.1.4. Evaluation of food viscoelastic behaviour

One of the most important challenges currently facing food producers is the evaluation of the structural properties of their foodstuffs (Kealy, 2006). The related texture is an important characteristic of food products because it directly influences the food sensory characteristics, playing a key role in the consumer choice and satisfaction (Mossaz et al., 2010). However, structural properties undergo important changes during storage and several instrumental techniques are developed to assess the structural time-dependent changes (Sheehan et al., 2005; Van Hekken et al., 2007). TPA and small amplitude oscillatory shear analysis have been used extensively to probe the mechanical and viscoelastic properties of mozzarella and stracchino cheeses, respectively (Ribero et al., 2007; Yu & Gunasekaran, 2005).

III.1.4.1. Determination of mechanical properties

The evaluation of food mechanical properties is generally performed using an instrumental texture profile analysis (TPA). It was developed in the 1960 to study the mechanical properties of foods and their relationship to the texture (Friedman et al., 1963; Szczesniak & Kleyn, 1963). Generally, TPA consists in two mechanical compressions of the sample (Fig. 37).

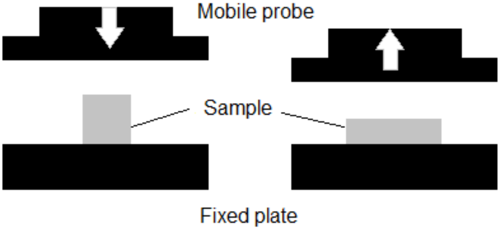


Figure 37. *A schematic representation of a TPA test.*

Fig. 38 shows a typical TPA force-time curve. Several parameters are quantified and their explications are following resumed (Bourne, 2002; Chen et al., 1979; Lee et al., 1978). In particular, hardness (g) is the peak force during the first compression cycle and it represents the maximum force required to compress the sample for the first time. Springiness (s) is the length to which the sample recovers in height during the time that elapses between the first compression cycle end the second compression cycle start; it is the ability of the sample to recover its original shape after first deforming force is removed. Gumminess (g) is defined as the product of hardness times cohesiveness and it represents the energy required to disintegrate a semisolid food to a state of readiness for swallowing. Chewiness (g) is defined as the product of hardness times cohesiveness times springiness and it represents the energy required to masticate a solid food product. Adhesiveness (g·s) is the area under the abscissa after the first compression and it is defined as the work necessary to pull the plunger away from the sample. Finally, cohesiveness (dimensionless) is obtained from the areas under the compression portion (downstroke) only and excludes the areas under the decompression portion (upstroke) instead of using the total area under positive force. It is the extent to which the sample could be deformed prior to rupture.

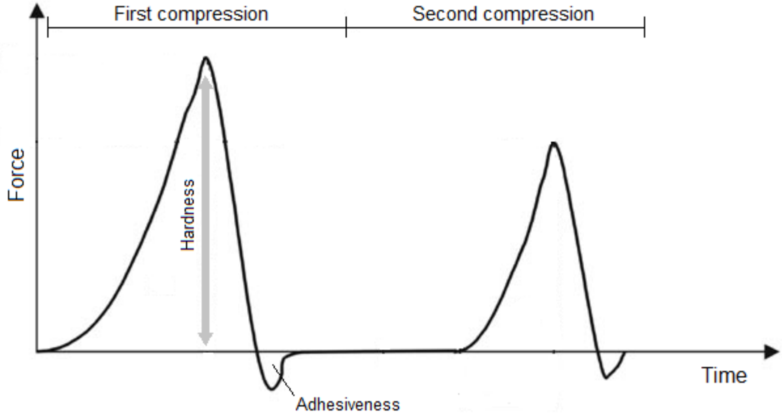


Figure 38. *A typical TPA force-time curve.*

III.1.4.2. Determination of rheological properties

Dynamic analysis is the most important method for determining rheological properties of viscoelastic foods (Tunick et al., 1995). Generally, the cheese is considered a viscoelastic material because it exhibits both elastic and viscous behaviours depending on the time scale of the deformation, which changes sinusoidally in time. In particular, during short time scales, the behaviour of cheese is mainly elastic. That is, a sample almost recovers its original form after the removal of the stress applied. However, at long time scales, the behaviour is mainly viscous and the most of the deformation remains after the removal of the stress applied (Ribero et al., 2007). In the small amplitude oscillatory shear analysis, a small strain is applied to the sample to measure the strength and flexibility of internal bonds and provides quantitative values for the viscoelastic properties of the cheese matrix (Diefes et al., 1993).

Fig. 39 shows the shear strain applied on a sample that follows the Eq. 1:

$$\gamma (t) = \gamma_0 \text{ sen } \omega t \qquad (1)$$

where γ_0 is the maximum amplitude of deformation and ω is the frequency.

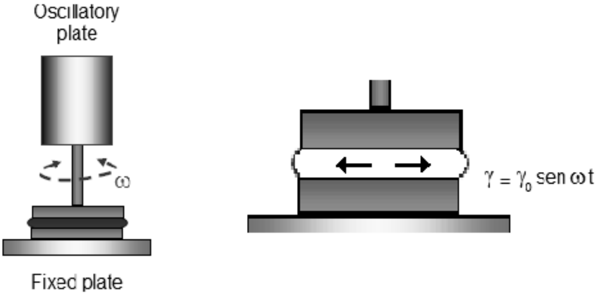


Figure 39. *A schematic representation of a sinusoidal deformation applied by a stress-controlled rheometer with parallel plates.*

Deriving Eq. 1 in function of time it is possible to obtain the deformation rate:

$$\dot{\gamma}(t) = \gamma_0 \, \omega \cos \omega t \qquad (2)$$

The resultant shear stress has a sinusoidal trend with amplitude τ_0 that has the same frequency but with a different angle δ from the deformation:

$$\tau(t) = \tau_0 \, \text{sen}\, (\omega t + \delta) \qquad (3)$$

The phase angle δ takes the following values:

- δ = 0° for an ideal solid: the strain is in phase with the oscillatory stress;
- δ = 90° for a Newtonian fluid: in this case there is a 90° phase lag of strain with respect to oscillatory stress;
- $0 < \delta < 90°$ for a viscoelastic material.

The stress can be developed as following:

$$\tau(t) = \tau_0 \cos \delta \, \text{sen}\, \omega t + \tau_0 \, \text{sen}\, \delta \cos \omega t \qquad (4)$$
$$\tau(t) = G' \gamma_0 \, \text{sen}\, \omega t + G'' \gamma_0 \cos \omega t \qquad (5)$$

The term ($\tau_0 \cos \delta \, \text{sen}\, \omega t$) is in phase with the strain and represents the elastic component, while ($\tau_0 \, \text{sen}\, \delta \cos \omega t$) is in phase with the strain rate and represents the viscous component.

The dynamic rheological data obtained through the small amplitude oscillatory shear analysis provide different viscoelastic parameters (Gunasekaran & Ak, 2003):

- $G' = \tau_0 / \gamma_0 \cos \delta$ represents the storage modulus, which is a measure of the energy stored and recovered per cycle of deformation;
- $G'' = \tau_0 / \gamma_0 \text{ sen } \delta$ represents the loss modulus, which is a measure of the energy dissipated per cycle of deformation;
- tan $\delta = G''/G'$ permits a discrimination between a solid-like (tan $\delta < 1$) and a liquid-like (tan $\delta > 1$) behaviour;
- $G^* = \sqrt{G'^2 + G''^2}$ is the complex shear modulus.

These viscoelastic parameters are fundamental to investigate the food structure. The dynamic moduli G' and G'' are dependent on frequency. Dilute polymeric solutions are characterized by $G'' > G'$, moreover at low frequency. Contrarily, hard gel systems have not dynamic modulus influenced by frequency and $G' > G''$.

III. EXPERIMENTAL SECTION

III.2. MATERIALS AND METHODS

III.2.1. Manufacture of food trays through lamination process

Three types of laminates (RIF, TOV and TOVA) were produced joining different films, such as PE, PS, OV and OVA, using a thermal lamination process. In particular:

- RIF laminate was constituted by PE/PS/PE films;
- TOV laminate was obtained from the lamination of PE/PS/OV films;
- TOVA was a laminate composed by PE/PS/OVA.

Thus, these laminates differed only for the film brought in contact with food that was PE, OV and OVA, respectively. The lamination process was conducted exploiting the similar heat sealing properties of the polymers that constituted the monolayer films joined. In this case, films passed through two heated rolls and their adhesion can be achieved by means of the pressure performed at their similar T_m (Tab. 1, p. 13), as shown in Fig. 40.

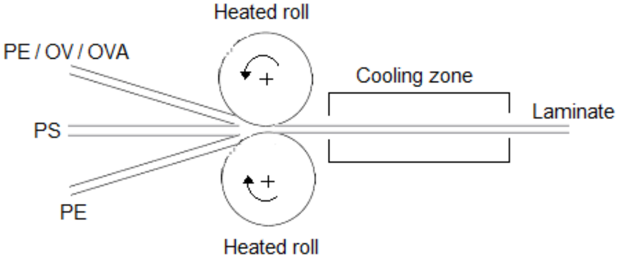


Figure 40. *A schematic representation of the lamination process used to obtain RIF, TOV and TOVA food trays.*

The laminates were then cooled and thermoformed to obtain RIF, TOV and TOVA food trays (Fig. 41). The thermoformed shape was chosen because it is typically used for the packaging of mozzarella cheese with conditioning brine.

The manufacture of food trays was kindly carried out by Sirap Gema (Sirap Group, Brescia, Italy).

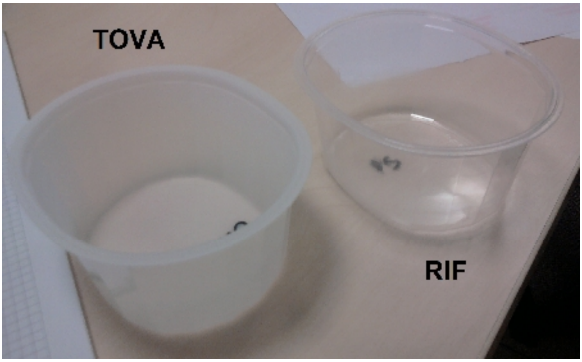


Figure 41. *Thermoformed TOVA and RIF food trays. Dimensions: L 11 × W 9 × H 5 cm.*

III.2.2. High-performance liquid chromatography (HPLC) active substance assay

III.2.2.1. Materials

Ammonium acetate (99.9% w/w purity) was purchased from Fluka (Fluka AG, Buchs, Switzerland). Glacial acetic acid and HPLC-grade acetonitrile were obtained from Carlo Erba (Carlo Erba Reagenti, Milan, Italy). Potassium sorbate (≥ 99% w/w purity) was purchased from Aldrich (Sigma-Aldrich, Seelze, Germany). High purity deionized water ($R > 18 M\Omega cm^{-1}$, $0.055\ \mu S cm^{-1}$, TOC < 5 ppb) was obtained with a Milli-Q Integral system (Millipore Co., Billerica, MA, USA).

The ultrasound treatment (UT) was performed using a VWR Ultrasonic Cleaner 900 D (VWR International, Milan, Italy) at 60°C for 2 h with an ultrasonic intensity of 600 W/cm^2 and a frequency of 45 kHz. A high-performance liquid chromatograph from JASCO (JASCO Inc., Easton, PA, USA) was used equipped as follows: 880-PU pump, 875-UV photodiode array detector and 880-02 unit ternary gradient.

III.2.2.2. Potassium sorbate extraction

Five OVA food trays (TOVA) were randomly chosen and internal surface calculated. According to the extraction ratio (1.25 mL of extracting solution for cm^2 of film) suggested by Buonocore et al. (2003), the internal surface of food trays was brought in contact with 150 mL of high purity deionized water. Then, all TOVA samples were subjected to UT, as described in the previous paragraph.

III.2.2.3. Mobile phase and standard preparation for HPLC assay

The mobile phase, consisted of 70% ammonium acetate buffer with 30% HPLC-grade acetonitrile, and the potassium sorbate standard were prepared as previously described in paragraphs I.2.3.3 and I.2.3.4 (p. 34).

III.2.2.4. Sample preparation for HPLC assay

TOVA samples were prepared by diluting 1 mL of potassium sorbate (SP) extract with 10 mL of mobile phase (dilution 1:10 v/v).

III.2.2.5. Quantitative determination of potassium sorbate by HPLC

The quantitative determination of potassium sorbate was conducted through HPLC according to Pylypiw & Grether (2000) and Buonocore et al. (2003), with minor modifications. The HPLC operating mode was isocratic, the injection volume was 20 μL and the column was at room temperature. The chromatography column was a Grace C-18 Reverse phase, 250×4.6 mm, 5 μm (Grace, Columbia, MD, USA). Sample data collection was optimized to 20 min per sample with UV detection at 255 nm. The optimal flow rate was determined to be 0.8 mL/min, according to Pylypiw & Grether (2000). The concentration of SP in the pieces of film evaluated was calculated by 7 point external standard method. The method had a detection limit of 0.1 mg/L.

The HPLC assay was carried out in the laboratories of the Department of Food Science (University of Udine).

III.2.3. Mozzarella cheese as real food matrix

III.2.3.1. Sample preparation

The small size pieces (125 g and 5-7 cm of diameter) of mozzarella cheese were manufactured using an industrial plant (Venchiaredo S.p.A., Pordenone, Italy). Formulation was as follows: milk, salt, rennet and starter cultures. Every small piece of mozzarella cheese was individually packaged in a single food tray containing 125 mL of conditioning brine and heat sealed with a transparent PET/PE top film (Opalen film, thickness 45 μm) kindly provided by Sirap Gema (Sirap Group, Brescia, Italy). All these operations were conducted directly in the aseptic room of Venchiaredo plant. The samples were firstly maintained for 8 hours at 4°C in a refrigerated open wall display (absorbed power: 1200 W) model Jolly 14 SL (Mondial Group, Alessandria, Italy) under internal lighting performed by four fluorescent vertical warm white lights (36 W, luminous efficacy: 3350 lm) model Lumilux Plus Eco Interna 41-827 (OSRAM AG, Munich, Germany). The daylight source was placed at about 20 cm over the samples. Then, all samples were maintained in an upright refrigerator (absorbed power: 506 W) model APSN 1060 (HIBER ALI S.p.A., Vittorio Veneto, Italy) under refrigerated conditions (4 ± 1°C; 50-60% R.H.).

For legal issues, we used only RIF and TOV as mozzarella cheese food packaging. In fact, also if the migration limit is respected and SP are listed into the community list of authorised substances established by EFSA, the release of the embedded SP in TOVA must comply with the international and national food additives legislation. As previously referred, the Italian law prohibits the use of SP in mozzarella cheese.

All analyses on food matrix were performed after 0, 8, 18 and 28 days of storage, in order to simulate a commercial storage time.

III.2.3.2. Colour measurements

The colour measurements were performed by means of a Minolta colorimeter, model CR-200 (Konica Minolta, Osaka, Japan), using a 1.5 cm diameter aperture. Colour was expressed as CIELab coordinates (CIE standard 6774K): in this system, L^* represents the colour lightness on a 0-100 point scale from black to white; a^* is the position between red (+) and green (-); b^* is the position between yellow (+) and blue (-). The colour intensity is expressed by a chroma value (C^*_{ab}) and its value was calculated according to the *formula*:

$$C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$$

The instrument was calibrated using a white colour tile standard ($L^* = 96.90$, $a^* = 0.14$, $b^* = 1.98$) and then ten measurements were performed directly on the surface of mozzarella cheese samples at ambient temperature.

III.2.3.3. Evaluation of pH

Mozzarella cheese (5 g) was added to 50 mL (ratio 1:10 w/v according to Brescia et al., 2005) of high purity deionized water (R > 18MΩcm^{-1}, 0.055 µScm^{-1}, TOC < 5 ppb) and then homogenated using a Polytron PT 3000 (Kinematica AG, Littau, Switzerland). The pH was evaluated on mozzarella cheese homogenates using a digital pH metre Hanna pH301 (Hanna Instruments, Milan, Italy).

III.2.3.4. Texture Profile Analysis (TPA)

Before the analysis, all cheese pieces packaged in the two different food packaging, were allowed to warm to ambient temperature until an equilibrium temperature (20°C) was reached. After the equilibration, samples were removed from the food packaging and four cylinders (1.5 cm high and 2 cm diameter) were prepared from every sample. The TPA was performed using a TA.XT*Plus* Stable Micro Systems Texture Analyser (Stable Microsystems Ltd., Surrey, England) coupled with the Texture Exponent 32 software. A double compression cycle test was performed up to 50% compression of the original height with an aluminium cylinder probe of 3.6 cm diameter. A time of 5 s was allowed to elapse between the two compression cycles. Force-time deformation curves were obtained with a 5 kg load cell applied at a cross-head speed of 5 mm/s. The following parameters were quantified: hardness (g), springiness (s), gumminess (g), chewiness (g), adhesiveness (g·s) and cohesiveness (dimensionless). TPA was performed at 0, 8, 18 and 28 days of storage and the values are the average of sixteen measurements from four samples.

All Texture Profile Analyses were performed in the laboratories of the Department of Food Science (University of Udine), under the supervision of Dr. Peressini.

III.2.3.5. Microbiological analyses

The samples of mozzarella cheese were subjected to microbiological analyses in order to monitor the dynamic changes in the microbial population responsible for the decrease of original mechanical properties of cheese and their hygienic quality. In particular, 25 g of each sample were transferred into a sterile stomacher bag and 225 mL of physiological solution (8 g/L NaCl, 1 g/L bacteriological peptone, Oxoid, Milan, Italy) were added and mixed in a Stomacher machine (PBI, Milan, Italy). Further decimal dilutions were made and the following analyses were carried out on triplicate agar plates: (a) total viable count on Gelatin Sugar Free Agar (Oxoid, Milan, Italy) incubated for 48-72 h at 30°C; (b) *Pseudomonas* spp. on Pseudomonas CFC Selective Agar (Oxoid, Milan, Italy) incubated at 25°C for 48 h; (c) Coliforms and *Escherichia coli* on Coli-ID medium (Bio Merieux, Marcy l'Etoile, France) incubated with a double layer at 37°C for 24-48 h; (d) Lactic Acid Bacteria (LAB) on MRS agar (Oxoid, Milan, Italy), modified by adding 0.025 g/L of Delvocid® Instant (DSM, Delft, The Netherlands), incubated with a double layer at 30°C for 48 h; (e) yeasts and moulds on Malt Extract Agar (Oxoid, Milan, Italy) supplemented with

tetracycline (1 g/L, Sigma, Milan, Italy) incubated at 25°C for 48-72 h. After counting, means and standard deviations were calculated.
These analyses were carried out in the laboratories of the Department of Food Science (University of Udine), under the supervision of Prof. Comi and Dr. Iacumin.

III.2.4. Evaluation of TiO_2 release in the mozzarella cheese conditioning brine

III.2.4.1. Chemicals and real samples

All the chemicals used were of analytical reagent grade (Merck Suprapure, Merck, Darmstadt, Germany) and were used as received. In all instances, high purity deionized water was used as the solvent. The reagents and the internal standards used for titanium metal analysis via ICP-MS are the following: nitric acid (HNO_3) 65% v/v (Merck Suprapur, Darmstadt, Germany), hydrogen peroxide (H_2O_2) 30% v/v (Merck Suprapur, Darmstadt, Germany), standard solution of Ti at the concentration of 0-1000 µg/L in HNO_3 5% v/v (Merck Suprapur, Darmstadt, Germany). Pure argon used for ICP determinations was of ultrapure grade (SIAD S.p.A., Bergamo, Italy).

III.2.4.2. Samples

The concentration of Ti in conditioning brine after 0, 8, 18, 28 days of storage at 4 ± 1°C was evaluated through ICP-MS. Analyzed samples are resumed in Tab. 7.

Table 7. *Codes of the conditioning brine samples.*

Sample	Days of storage
S1	0
S2	8
S3	18
S4	28

III.2.4.3. Sample microwave digestion

All microwave digestions were run on a Milestone MLS-1200 MEGA (FKV, Bergamo, Italy) equipped with an EM-45 exhauster of nitric acid fumes, a control panel and a MDR-1000/6/100/110 rotor, this last being a turntable operating with a maximum of six digestion vessels. Each digestion vessel consisted of a tetrafluoromethoxyl polymer (TFM) sample holder inserted in a hollow polyether-ether-ketone copolymer (PEEK) container. At the top of each vessel, a PEEK relief-valve was present, aimed to allow vapour release for pressure values exceeding 110 bars. The three step power program adopted for the microwave digestion was as follows: (I) 3 min with an irradiation power of 250 W; (II) 3 min with 400 W; (III) 3 min with 600W; (IV) 3 min with 700 W. 5 mL of every sample were introduced in a digestion vessel and added with 4 mL of HNO_3 and 0.5 mL of H_2O_2. After microwave mineralisation, the digestion vessels were cooled and their content was transferred into 100 mL glass volumetric flasks where digested samples were diluted with ultrapure water, thus achieving the solutions suitable for subsequent analysis.

III.2.4.4. Instrumentation, operative conditions and procedure

ICP-MS measurements on microwave digested samples were performed by a Spectromass 2000 Type MSDIA10B (Spectro Analytical Instruments, Kleve, Germany) under the operating conditions reported previously, according to Tubaro et al. (1999), Di Narda et al. (2001) and Tubaro et al. (2002). All measurements were performed after calibrating these operating conditions, so as to maximize the signal with respect to the background noise for m/z equal to 48 (Ti isotope) amu. ^{48}Ti was exploited because it is the major isotope in terms of abundance (73.8%). The nebulization system consisted of a conventional concentric flow pneumatic nebulizer (Meinhard type, Spectro Analytical Instruments, Kleve, Germany) fed with a nebulizer flow of argon of 1.1 L/min which led to a nebulizer gas pressure of 2.8 bars. Mean values and standard deviations relative to 10 replicate measurements were always employed. The signals recorded were related to Ti concentrations by resorting to a suitable calibration plot. Under these conditions, the response was verified to be linear in the range 0-1000 µg/L of titanium with a correlation coefficient of 0.998. The coefficient of variation was equal or lower than 9% (10 replicate measurements).

The sample microwave digestion and all analyses were carried out in the laboratories of the Department of Chemical Sciences (University of Udine), under the supervision of Prof. Tubaro.

III.2.5. Stracchino cheese as real food matrix

III.2.5.1. Sample preparation

The small size cubes (100 g) of stracchino cheese were manufactured using an industrial plant (Venchiaredo S.p.A., Pordenone, Italy). Formulation was as follows: milk, starter cultures, salt and rennet. Every small cube of stracchino cheese was individually packaged in each single food tray (RIF, TOV and TOVA) and heat sealed with a transparent PET/PE top film (Opalen film, thickness 45 μm) kindly provided by Sirap Gema (Sirap Group, Brescia, Italy). All these operations were conducted directly in the aseptic room of Venchiaredo plant. The samples were firstly maintained for 8 hours at 4°C in a refrigerated open wall display (absorbed power: 1200 W) model Jolly 14 SL (Mondial Group, Alessandria, Italy) under internal lighting performed by four fluorescent vertical warm white lights (36 W, luminous efficacy: 3350 lm) model Lumilux Plus Eco Interna 41-827 (OSRAM AG, Munich, Germany). The daylight source was placed at about 20 cm over the samples. Then, all samples were maintained in an upright refrigerator (absorbed power: 506 W) model APSN 1060 (HIBER ALI S.p.A., Vittorio Veneto, Italy) under refrigerated conditions (4 ± 1°C; 50-60% R.H.). All analyses on food matrix were performed after 0, 9, 15, 21 and 28 days of storage, which corresponds to a commercial storage time.

III.2.5.2. Evaluation of pH

Stracchino cheese (5 g) was blended in 50 mL (ratio 1:10 w/v according to Brescia et al., 2005) of high purity deionized water ($R > 18 M\Omega cm^{-1}$, 0.055 $\mu S cm^{-1}$, TOC < 5 ppb) and then homogenated using a Polytron PT 3000 (Kinematica AG, Littau, Switzerland). The pH was evaluated by means of a digital pH metre Hanna pH301 (Hanna Instruments, Milan, Italy).

III.2.5.3. Rheological analyses

Before the analysis, all cheese cubes packaged in the different food trays were allowed to warm to ambient temperature until an equilibrium temperature (20°C) was reached, in order to have the same temperature of the test cell. The rheological measurements were performed using a controlled stress rheometer (SR5, Rheometric Scientific, Piscataway, NJ, USA) equipped with parallel plates (25 mm diameter, 2 mm gap). A small amount of stracchino cheese sample was placed between the plates of the rheometer and excess cheese was carefully trimmed. Stracchino cheese sample was rested 5 min after loading before testing. A preliminary stress sweep test at 1 Hz was performed to determine linear viscoelastic range at 20°C. This was followed by a frequency sweep test in linear viscoelastic region from 0.1 to 10 Hz at 20°C. Data obtained were storage modulus (G'), loss modulus (G''), tan δ (G''/ G') and complex modulus (G^*). Statistical comparisons were made at 1 Hz. The rheological measurements were performed in triplicate for each food tray (RIF, TOV and TOVA) at 0, 9, 15, 21 and 28 days of storage.

All rheological analyses were carried out in the laboratories of the Department of Food Science (University of Udine), under the supervision of Dr. Peressini.

III.2.5.4. Microbiological analyses

The samples of stracchino cheese were subjected to microbiological analyses in order to monitor the dynamic changes in the microbial population responsible for the decrease of original rheological properties of cheese and their hygienic quality. Analyses were the same conducted on mozzarella cheese samples (paragraph III.2.3.5, p. 92): 25 g of each sample were transferred into a sterile stomacher bag and 225 mL of physiological solution were added and mixed in a Stomacher machine (PBI, Milan, Italy). Further decimal dilutions were made and the following analyses were carried out on triplicate agar plates: (a) total viable count; (b) *Pseudomonas* spp.; (c) Coliforms and *Escherichia coli*; (d) LAB and (e) yeasts and moulds. Data are reported as mean ± standard deviation.

These analyses were carried out in the laboratories of the Department of Food Science (University of Udine), under the supervision of Prof. Comi and Dr. Iacumin.

III.3. RESULTS AND DISCUSSION

In the first chapter, we have concluded that OV film seemed to be a good carrier of preservatives because the resultant OVA film presented available potassium sorbate (SP) on the film surface. However, the SP distribution was scarcely homogeneous and the recovering data ranged from 5 to 22% of the initial amount of SP blended with pellets during extrusion process. Thus, to assess the effects of packaging on the food preservation, we have been proposed to laminate two films of heat sealable polyolefins, such as PE and SP, to OV and OVA films, respectively, by dry lamination in order to provide higher barrier properties and experimental reproducibility. The obtained TOV and TOVA food trays confirmed that produced composite films are able to give a stable white opaque tone, as shown in Fig. 41 (p. 89). Contrarily, RIF showed a total transparency.

III.3.1. Evaluation of preservative presence and distribution in OVA food tray

It is well known that dry lamination process relies on the similar sealing point of the films involved in the laminate production (Kirwan & Strawbridge, 2003; Piergiovanni & Limbo, 2010b). In fact, films are approached to their T_m passing through heated rolls. Clearly, this additional thermal stress may induce the loss of low-melting components of film, such as the added preservative in the OVA case (Alvarez, 2000; Flores et al., 2010; Hotchkiss, 1997; Kim et al., 1999). Thus, to confirm the previous results and to obtain more insight about the SP distribution into TOVA, a HPLC assay was developed.

In our work, we analyzed 5 different TOVA performing an UT assisted extraction of sorbate agent using water as extracting solvent. The UT assisted extraction was optimized following the same parameters performed for OVA films. Also in this case, water extracts were investigated using a HPLC analysis according to Pylypiw & Grether (2000) and Buonocore et al. (2003), with minor modifications, and the sorbate concentration was calculated by 7 point external standard method. Theoretically, each water extract can have 30 mg/L of SP (corresponding to 4.6 mg of SP into each investigated food tray with a composite material mass of 0.66 g) in case of total active agent extraction. Using the linear response of the SP calibration showed in Fig. 19 (p. 45), we were able to confirm that the SP distribution was scarcely homogeneous, with significant differences between TOVA food trays. Furthermore, the recovering data ranged from 3 to 11%, where % w/w was expressed as fraction of the initial amount of SP blended with pellets. All results are reported in Tab. 8.

Table 8. *Potassium sorbate concentration for each water extract expressed in mg/mL and % w/w. Values followed by different letter are significantly different at the p-level of 5 %.*

Sample	[SP] in water extract sample (mg/mL)	SP in sample* (%)
TOVA1	0.95 ± 0.11^{d}	3.2
TOVA2	3.21 ± 0.30^{a}	10.7
TOVA3	1.02 ± 0.13^{d}	3.4
TOVA4	1.50 ± 0.15^{c}	5.0
TOVA5	2.61 ± 0.25^{b}	8.7

* ([ ] extract·100)/[ ] theoretical
Data are mean ± standard deviation

These evidences reveal that only a small amount of the initial added SP keep available also at the TOVA surface, but the latter is lower than that available at the OVA film surface only (Tab. 6, p. 46). This additional loss of SP may be ascribed to loose occurring during dry lamination process, which can determine thermomechanical stress on laminated substrates, according to Kim et al. (1999) and Kirwan & Strawbridge (2003).

III.3.2. Mozzarella cheese as real food matrix

The Italian Ministerial Decree of 27 February 2008, which has implemented the Italian Ministerial Decree No 209 of 27 February 1996, establishes that in the mozzarella cheese the use of SP is forbidden. As a consequence, cheese samples were voluntary not packaged into TOVA. Thus, only TOV effectiveness was compared with a usual commercial food tray, such as RIF. In our work, a small piece of mozzarella cheese was packaged with a specific volume of conditioning brine and different evaluations, such as colour and pH measurements, TPA and microbiological analyses were performed on the cheese samples after 0, 8, 18 and 28 days of storage at 4°C.

III.3.2.1. Evaluation of surface colour changes during storage time

Colour parameters of the surface mozzarella cheeses packaged into RIF and TOV at different storage times are shown in Tab. 9. Mozzarella cheese samples exhibited a slight decrease in the L^* and a^* values with the increase in storage time. Contrarily, b^* and C^*_{ab} values revealed a marked increase. The decrease in the whiteness of cheese during storage is probably associated with increased protein hydration, which reflects a decrease in the number of free moisture droplets and thereby a reduced degree of light scattering, according to Rudan et al. (1998) and Sheehan et al. (2005). Thus, mozzarella cheese aging resulted in a slight significant reduction in whiteness and increase in yellowness and chroma value. However, these slight changes in colour parameters are not much evident for human sight, as shown in Fig. 42.

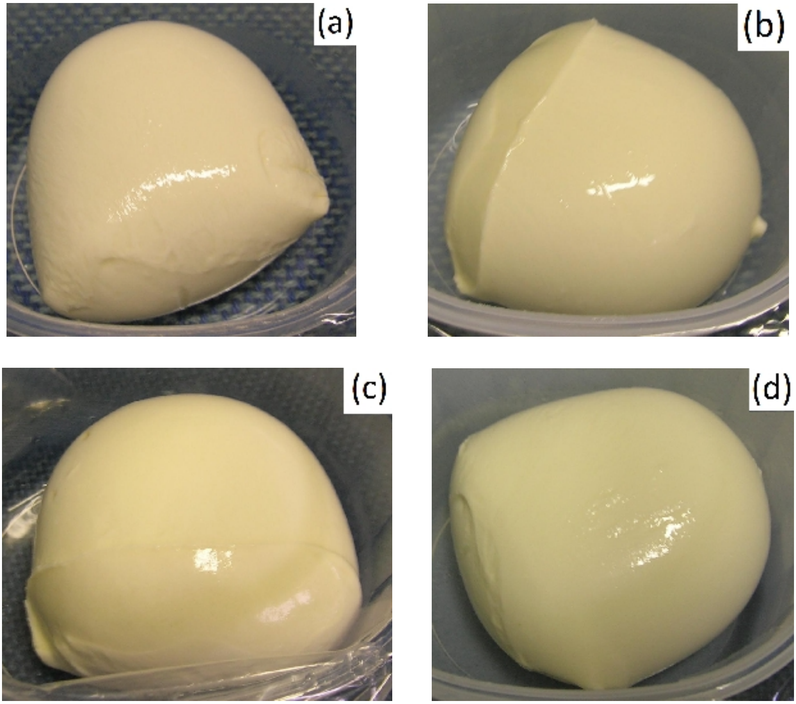


Figure 42. *Mozzarella cheese samples packaged both into RIF after 0 (a) and 28 days (c) of storage and into TOV after 0 (b) and 28 days (d) of storage.*

Furthermore, there are no significant differences between food trays effectiveness in terms of colour maintenance. Independently by food trays used, the surface of mozzarella cheese became less white and more yellow during the storage time.

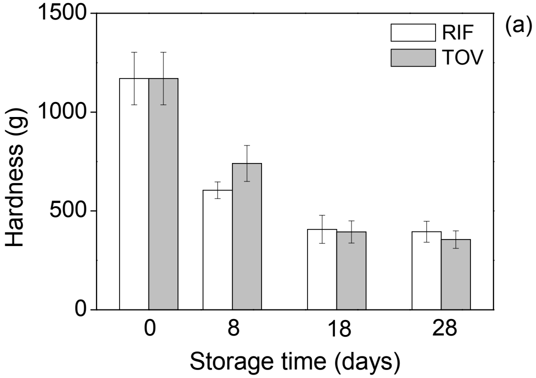
Table 9. *Effects of storage time on changes in colour parameters of the surface cheese samples. Values in the same row followed by different letter are significantly different at the p-level of 5 %.*

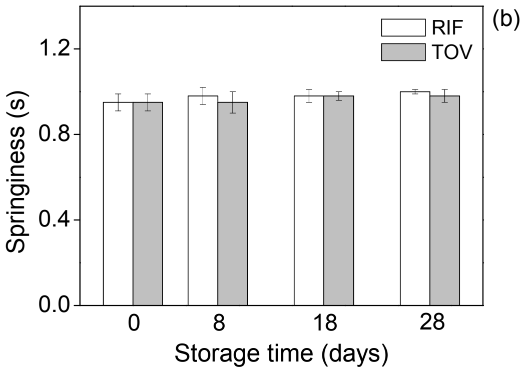
Colour parameter	Packaging	0 days	8 days	18 days	28 days
L^*	*RIF*	89.2 ± 0.3^a	88.7 ± 0.3^b	88.1 ± 0.3^b	87.2 ± 0.2^c
	TOV	89.2 ± 0.3^a	88.8 ± 0.3^b	88.2 ± 0.4^b	87.4 ± 0.5^c
a^*	*RIF*	2.4 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
	TOV	2.4 ± 0.1	2.2 ± 0.1	2.3 ± 0.2	2.2 ± 0.2
b^*	*RIF*	6.9 ± 0.3^b	7.6 ± 0.2^{ab}	8.0 ± 0.2^a	8.3 ± 0.2^a
	TOV	6.9 ± 0.3^b	7.6 ± 0.3^{ab}	8.1 ± 0.2^a	8.4 ± 0.2^a
C^*_{ab}	*RIF*	27.1 ± 2.0^c	31.2 ± 1.7^b	33.0 ± 1.7^{ab}	35.2 ± 1.9^a
	TOV	27.1 ± 2.0^c	31.5 ± 2.0^b	33.2 ± 2.2^{ab}	35.9 ± 2.0^a

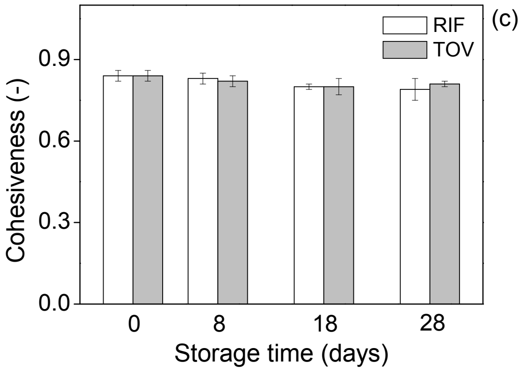
Data are mean ± standard deviation

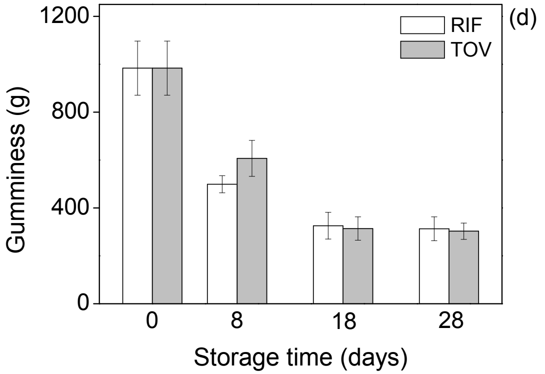
III.3.2.2. Evaluation of mechanical properties during storage time

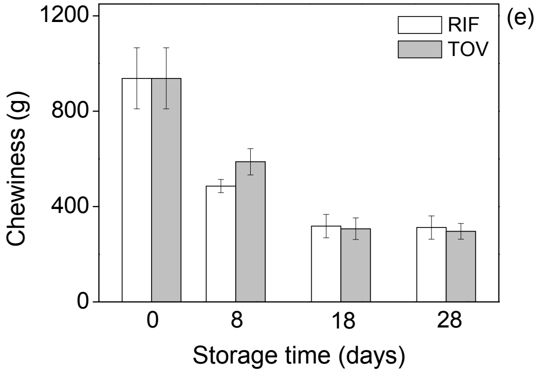
Texture is widely regarded as an important attribute for product acceptability together with surface colour. These aspects play the most important role in consumer perceptions of quality (Bourne, 2002; Kealy, 2006; Monaco et al., 2008). Sensory textural properties of cheese are related to how the food is perceived when chewing and swallowing. As a consequence, the mechanical properties of cheese are the fundamental component of sensory texture (Foegeding et al., 2003). In our work, the evaluation of mechanical properties during storage time was investigated using TPA. This analysis was performed on mozzarella cheese sample with the aim to simulate the chewing process in mouth. The TPA responses showed that the aging of the cheese for 28 days altered the hardness, gumminess and chewiness of mozzarella cheese, as shown in Fig. 43.




(b)
RIF
TOV
Springiness (s)
0.0
0.4
0.8
1.2
0
8
18
28
Storage time (days)


(c)
RIF
TOV
Cohesiveness (-)
0.0
0.3
0.6
0.9
0
8
18
28
Storage time (days)


(d)
RIF
TOV
Gumminess (g)
0
400
800
1200
0
8
18
28
Storage time (days)



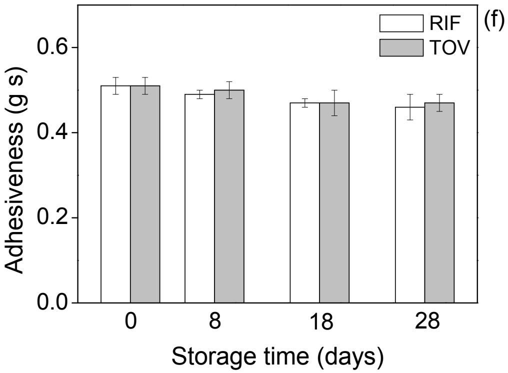


Figure 43. *Texture profile analysis parameters [hardness (a), springiness (b), cohesiveness (c), gumminess (d), chewiness (e), adhesiveness (f)] of mozzarella cheeses, packaged into RIF and TOV, and stored at 4°C.*

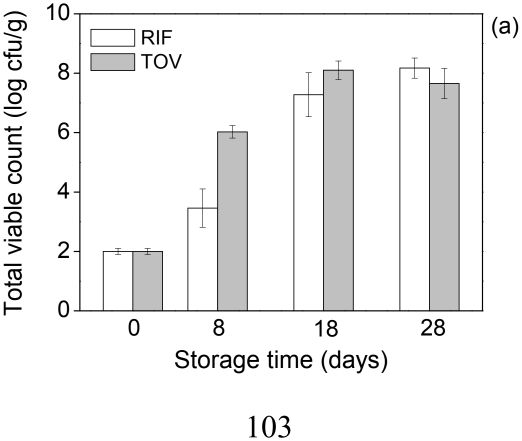
The general trend was a significant decrease of these TPA parameters during storage time, independently by food trays used. Springiness, cohesiveness and adhesiveness did not change significantly with the increase in storage time. It is evident that all parameters referred to the ability to recover a deformation force, such as springiness or cohesiveness, have not presented significant changes during storage time. Contrarily, the parameters that represented the force to compress or disintegrate the sample, such as the hardness, gumminess or chewiness, are significantly influenced by storage time. This behaviour was attributed by Messens et al. (2000) to a weakening of protein network interactions. Finally, TOV have not showed higher effectiveness than RIF and, as a consequence, the use of RIF or TOV as food tray for package mozzarella cheese and its conditioning brine seems to be not effective to increase the maintenance of the texture.

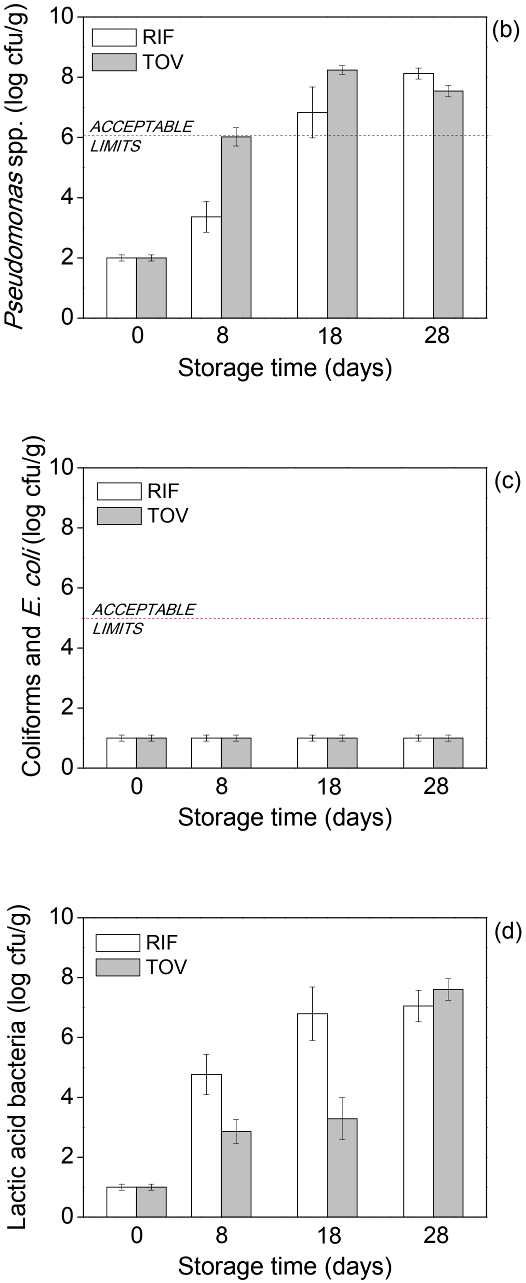
III.3.2.3. Evaluation of microbial population changes during storage time

The shelf-life of mozzarella cheese has been attributed above all to microbiological spoilage. This spoilage is often caused by the growth of *Pseudomonas* spp., coliforms and/or by psychrotrophic bacteria that grow on the cheese surface (Cantoni et al., 2003; Rondinini & Garzaroli, 1990). The significance of *Pseudomonadaceae* and other psychrotrophic bacteria on cheese is well known. Their contamination is a major concern for the Italian product because they could affect the odour within the storage time (Bevilacqua et al., 2007).

Fig. 44 shows the evolution of different microbial population over storage time. As expected, *Pseudomonas* spp. and LAB increased in the analyzed samples and, as a consequence, also total viable count followed the same trend. However, the growth of *Pseudomonas* spp. and LAB presents different evolutions referred to the food tray used as packaging. Contrarily, the experimental conditions inhibited the growth of coliforms, yeasts and moulds.

During the first 8 days of storage, the growth of *Pseudomonas* spp. (Fig. 44b) was significantly higher in mozzarella cheese samples packaged into TOV. Moreover, the microbial count of these samples equalized already the limit of acceptability, while this limit was achieved by RIF samples at 18 days of storage.




Pseudomonas spp. (log cfu/g)
10
8
6
4
2
0
RIF
TOV
ACCEPTABLE LIMITS
(b)
0
8
18
28
Storage time (days)
Coliforms and E. coli (log cfu/g)
10
8
6
4
2
0
RIF
TOV
ACCEPTABLE LIMITS
(c)
0
8
18
28
Storage time (days)
Lactic acid bacteria (log cfu/g)
10
8
6
4
2
0
RIF
TOV
(d)
0
8
18
28
Storage time (days)

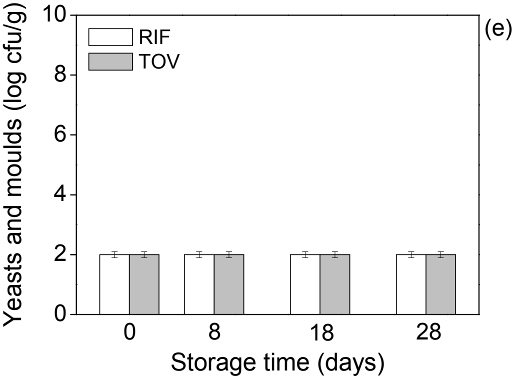


Figure 44. *Evolution of different microbial population [total viable count (a), Pseudomonas spp (b), coliforms and E. coli (c), LAB (d), yeasts and moulds (e)] of mozzarella cheeses, packaged into RIF and TOV, stored at 4°C. The acceptable limits are adopted according to Sinigaglia et al., 2008.*

All samples at 18 days exceeded the break-point of *Pseudomonas* spp., set to $1 \cdot 10^6$ cfu/g (Sinigaglia et al., 2008), and, for this reason, they may be considered "not acceptable" at 18 days of storage. Above these assumptions, it is evident that TOV permits a major growth of *Pseudomonadaceae*. This behaviour may be due to inhibition of LAB growth for mozzarella cheese samples packaged into TOV. These bacteria are gram positive, non-spore forming and naturally present in foodstuffs. Generally, they have no respiratory system and rely mostly on fermentative metabolism. This behaviour has been used for food production and preservation because they cause rapid acidification of organic substrates through the production of either a lactic acid, carbon dioxide, acetic acid and ethanol (heterofermentation) or entirely lactic acid (homofermentation) as the major metabolic end-products. As a consequence, these metabolic compounds decrease the pH of food low enough to inhibit the growth of several microorganisms, including the most common human pathogens (Ali, 2010; Caplice & Fitzgerald, 1999; McKay & Baldwin, 1990; Nuñez et al., 1985). Observing Fig. 44d, it is clear that the LAB growth rate of mozzarella cheese samples packaged into TOV is significantly lower than cheese samples packaged into RIF until the eighteenth day of storage. During this time it is possible to observe a higher increase of the total viable count in the samples packaged into TOV. When the presence of LAB increases between 18-28 days of storage, then the total viable count showed only a slight decrease that may be due to a higher acidification of the food matrix. This assumption was confirmed by the pH of samples reported in Tab. 10.

Table 10. *pH evolution of mozzarella cheese samples stored at 4°C and packaged into RIF and TOV. Values in the same row followed by different letter are significantly different at the p-level of 5 %.*

Packaging	**0 days**	**8 days**	**18 days**	**28 days**
RIF	5.6 ± 0.0^a	5.4 ± 0.1^b	5.0 ± 0.1^c	5.0 ± 0.1^c
TOV	5.6 ± 0.0^a	5.6 ± 0.0^a	5.4 ± 0.1^b	5.0 ± 0.1^c

Data are mean ± standard deviation

The cheese acidification was more quickly when samples were packaged into RIF. As a result, from 0 to 18 days of storage, the growth rate of *Pseudomonas* spp. decrease in cheese samples packaged into RIF where the LAB growth is significantly higher. Contrarily, when the growth rate of LAB increases and the food matrix is more acidified, then the growth of *Pseudomonas* spp. is significantly reduced also in the cheese samples packaged into TOV, according to the work of Carraro et al. (2011) on the LAB influence against the growth of *Pseudomonas* spp.

The observed lower growth of LAB into TOV samples may be due to the alkaline character referred to OV film. As described previously, the aqueous extract of OV film showed alkaline pH around 10 and this behaviour could influence the interface between food matrix and packaging. Considering that the growth of LAB may be inhibited at pH values > 9 (Knittel, 1965; Lancefield, 1933), it is possible to infer that the particular property of OV film may influence the growth rate of LAB.

III.3.3. Evaluation of TiO_2 release in the mozzarella cheese conditioning brine

As presented in the first chapter, all materials intended to come into contact with food have to respect a migration limit defined into the Council Regulation (EC) No 10/2011 (Commission of the European Communities, 2011). Also if the migration limit is respected, a release of substances from the packaging to the food can happen. Titanium dioxide is a typical case of "dual use" substance. In fact, it can be used both as plastic material additive (Fassina, 2008) and as food colorant (Commission of the European

Communities, 2008). However, the Italian Ministerial Decree of 27 February 2008 establishes that the use of TiO_2 is forbidden in mozzarella cheese formulations. Considering that the laminated OV film has a composite formulation where TiO_2 is included as a white pigment, the release of the embedded TiO_2 from TOV to the mozzarella cheese or the conditioning brine has to be avoided.

To assess the eventual migration of this mineral into the mozzarella cheese conditioning brine, the ICP-MS analysis of Ti ions was performed periodically during storage time. Results are shown in Tab. 11.

Table 11. *Concentration (μg/mL) of Ti metal in the conditioning brines.*

Sample	Storage time (days)	[Ti] in conditioning brine (μg/mL)
S1	0	5.3 ± 0.1
S2	8	5.4 ± 0.1
S3	18	5.6 ± 0.1
S4	28	5.6 ± 0.1

Data are mean ± standard deviation

As observed, considering the detection limits of the instrumental technique, in all of the analysed samples Ti were found in the interval between 5.3 and 5.6 μg/mL. Comparing the Ti levels at time 0 and after 28 days, it is possible to establish that TiO_2 is not migrated from the packaging to the mozzarella cheese conditioning brine during storage time. As a consequence, TOV can be used as food packaging for Italian mozzarella cheese. Finally, the detected Ti level is probably due to the metal level in drinking-water, that Cerruti (2006) has valued at about 5-10 μg/mL.

III.3.4. Stracchino cheese as real food matrix

As reported in the General section, stracchino is an Italian traditional cheese characterized by a gel-like structure (Belitz et al., 2009). Generally, stracchino and other cheeses are considered viscoelastic foods because they exhibit both elastic and viscous behaviour depending on the time scale of the deformation. Structural characteristics of cheese affect its viscoelastic behaviour and it undergoes notable changes during storage time (Subramanian & Gunasekaran, 1997).

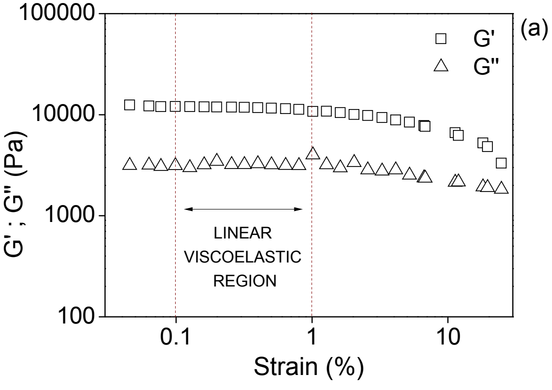
In our work, a small amplitude oscillatory tests and microbiological analyses were performed periodically during storage time in order to evaluate the TOV and TOVA effectiveness compared with a usual commercial food tray, such as RIF. Analyses were carried out on the cheese samples after 0, 9, 15, 21 and 28 days of storage at 4°C.

III.3.4.1. Evaluation of rheological properties during storage time

As previously described, dynamic rheological measurements are useful to study cheese viscoelastic behaviour. In this way, a small strain was applied to the sample to measure the strength and flexibility of internal bonds and provides quantitative values for the viscoelastic properties of the stracchino cheese matrix.

A preliminary stress sweep test at 1 Hz frequency was performed to determine linear viscoelastic range at 20°C. In this range the viscoelastic parameters, such as *G′* and *G″*, are independent by the applied strain (Gabriele et al., 2004). This test was followed by a frequency sweep test carried out in the previously determined linear viscoelastic region. The data obtained included the two components of complex shear modulus (G^*): the storage modulus (*G′*), which represent the elastic component and the loss modulus (*G″*), which mean the viscous component (Gunasekaran & Ak, 2003).

It is evident that the linear viscoelastic region for stracchino cheese is ranged between 0.1-1.0% of strain (Fig. 45a). Thus, the frequency sweep test was applied with a constant % of strain included in the linear viscoelastic region previously investigated. Fig. 45b shows that *G′* was greater than *G″*, revealing that analyzed stracchino cheese exhibits a gel-like behaviour (Gabriele et al., 2004).



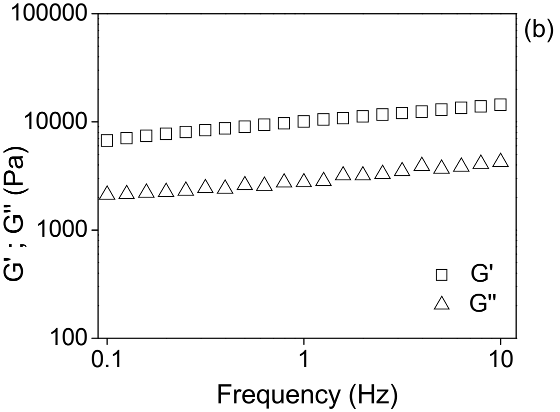


Figure 45. *Stress sweep test (a) and frequency sweep test (b).*

Also the loss tan δ (G''/G') confirmed a solid-like behaviour of cheese with a value of 0.27 at time 0. Fig. 46 and 47 presents the general trends both of the storage modulus (G') and of the loss tan δ at 1 Hz during storage for stracchino cheese samples packaged into the different food trays.

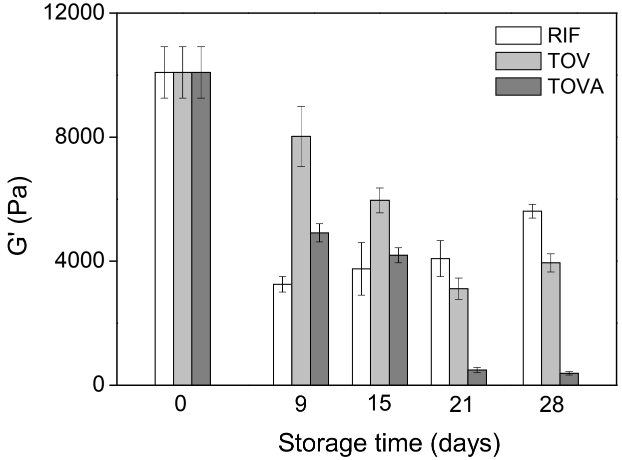


Figure 46. *Evolution of the storage modulus (G') at 1 Hz of stracchino cheeses, packaged into RIF, TOV and TOVA, stored at 4°C.*

The storage modulus (G'), which is referred to the interactions number that contributed to system elasticity, presents a significant decrease within the storage time. Samples in TOV and TOVA shows a progressive reduction of this modulus, while samples in RIF are primary characterized by a rapid reduction in the first 9 days of storage. Then, G' reveals a progressive increase. The progressive reduction of G' within the storage time may be referred to a loss in gel structure, while the particular trend presented by samples packaged in RIF may be due to a syneresis (Faers et al., 2006; Ramirez-Santiago et al., 2010). However, stracchino cheese packaged in TOV exhibited the lowest decrease in the storage modulus among the samples. These findings were confirmed also by trend of tan δ (Fig. 47): samples in TOV presented a greater maintenance of solid-like behaviour during the storage, while stracchino cheese samples in TOVA tend to exhibit liquid-like behaviour increasing the time of storage.

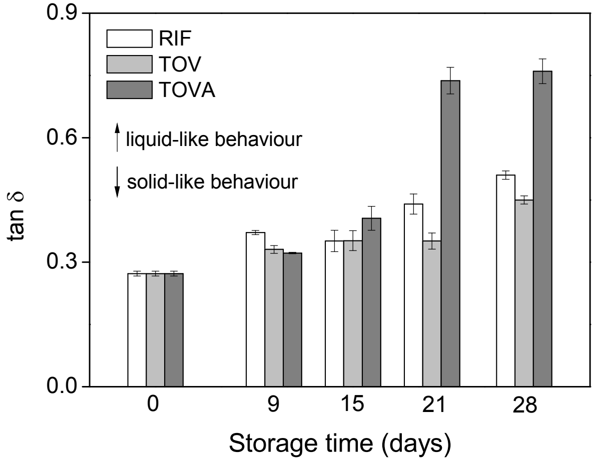


Figure 47. *Evolution of the loss tan δ at 1 Hz of stracchino cheeses, packaged into RIF, TOV and TOVA, stored at 4°C.*

In order to perform a complete characterization of stracchino cheese samples it is very important to obtain a measure of its consistency (Curcio et al., 2001). Considering Log G' and G'' vs. Log frequency showed a linear behaviour and that tan $\delta < 1$, it is possible to apply the weak gel model according to Gabriele et al. (2001). With this model the strength of the network (A) and the coordination number (z) were determined according to the following equation (Eq. 6):

$$G^* = A \cdot \omega^{1/z} \qquad (6)$$

Fig. 48 and 49 resumes the values of these two parameters, obtained by fitting the experimental results by Eq. 6.

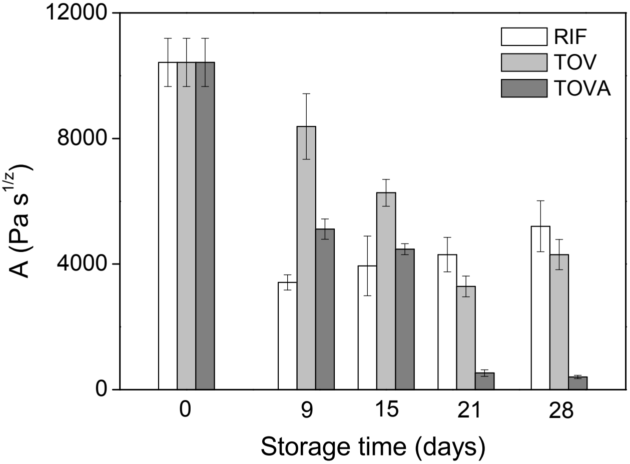


Figure 48. *Evolution of the network strength of stracchino cheeses packaged into RIF, TOV and TOVA, stored at 4°C.*

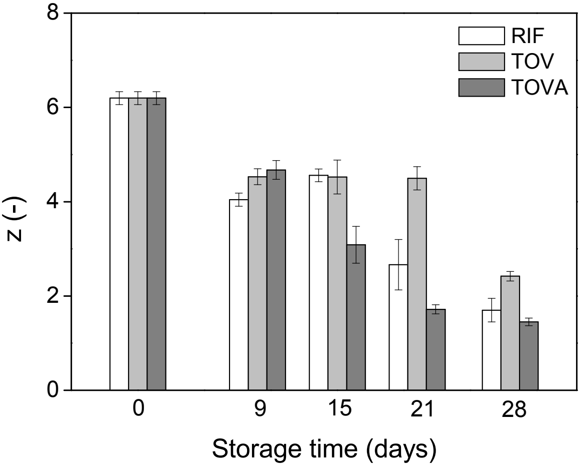
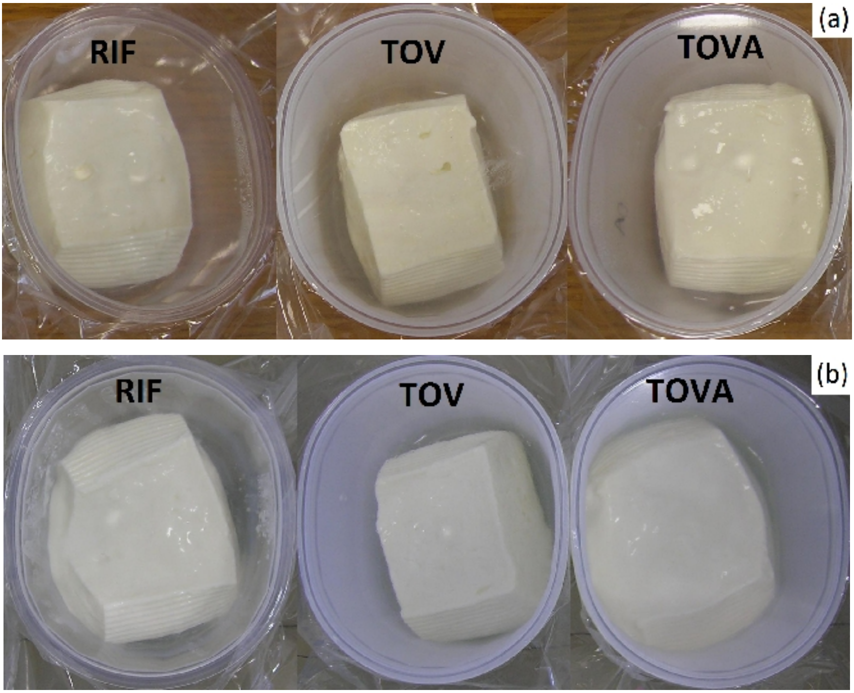


Figure 49. *Evolution of the coordination number of stracchino cheeses packaged into RIF, TOV and TOVA, stored at 4°C.*

It appears that samples in TOVA have the higher decrease rate of A and z parameters. According to the weak gel model, this indicates a low structured stracchino cheese samples and it was confirmed by the higher liquid-like behaviour shown in frequency sweep test by the same samples packaged in TOVA. Contrarily, stracchino cheese samples in TOV have evidenced a greater maintenance of network strength and coordination number during the storage time. Finally, the weak gel model has confirmed that stracchino cheese samples packaged into RIF have a different trend in terms of elastic behaviour loss (rapid reduction in the first 9 days of storage). The previous assumption based on a lower hydration of food matrix is supported by z value trend. In fact, also if the strength of the network seems to increase after 9 days of storage, the coordination number suggests that the gel structure is slightly loss and the increase of network strength is due to a decrease in matrix moisture content.

Structure changes displayed by rheological measurements were evident also observing Fig. 50. As we can see, stracchino cheese samples that have maintained their original structure are those packaged into TOV, while TOVA seems to be the worst food packaging in terms of maintenance of food structural characteristics.



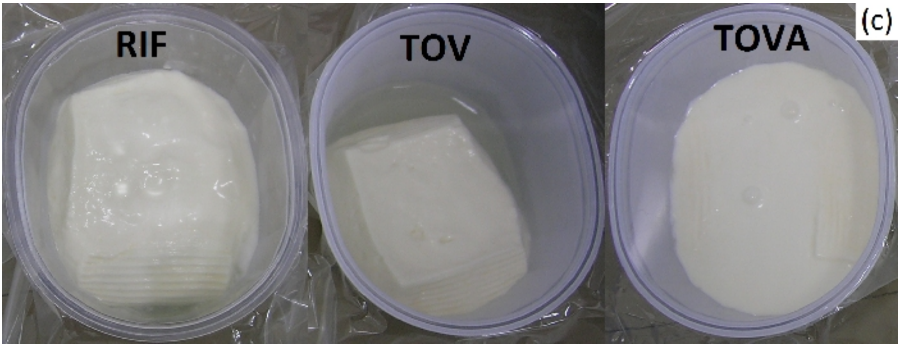
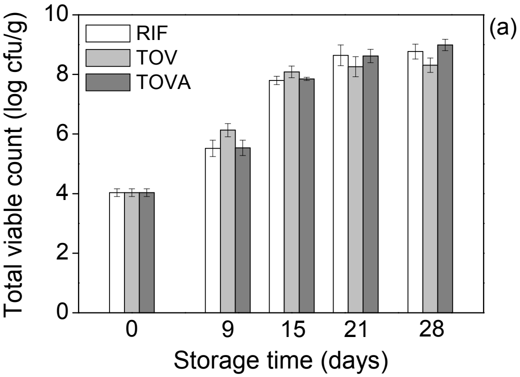


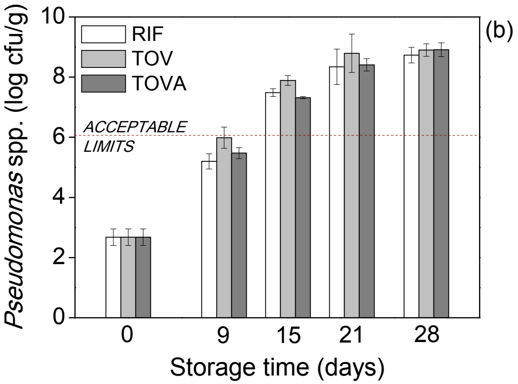
Figure 50. *Stracchino cheese samples packaged both into RIF, TOV and TOVA after 9 (a), 15 (b) and 21 days (c) of storage at 4°C.*

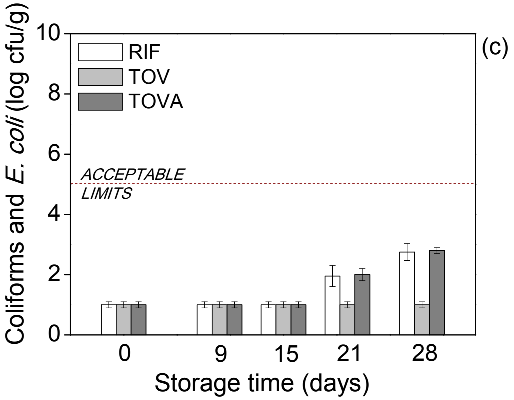
III.3.4.2. Evaluation of microbial population changes during storage time

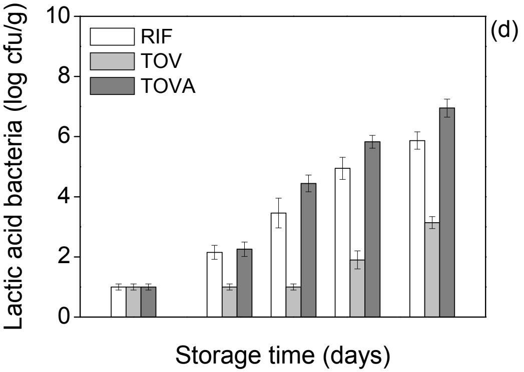
As previously described, the shelf-life of cheese has been attributed above all to microbiological spoilage. This spoilage is manifested by the presence of a wide variety of metabolic by-products, causing off-odours, off-flavours and visible changes in colour or texture (Ledenbach & Marshall, 2010).

In our work on stracchino cheese, structural changes within storage time are evident. To evaluate the role of microbial population on cheese aging, additional microbiological analyses were carried out. Fig. 51 shows the evolution of different microbial population during storage time. As expected, the total viable count increased significantly in the analyzed samples.




(b)
Pseudomonas spp. (log cfu/g)
10
8
6
4
2
0
RIF
TOV
TOVA
ACCEPTABLE
LIMITS
0
9
15
21
28
Storage time (days)


(c)
Coliforms and E. coli (log cfu/g)
10
8
6
4
2
0
RIF
TOV
TOVA
ACCEPTABLE
LIMITS
0
9
15
21
28
Storage time (days)


(d)
Lactic acid bacteria (log cfu/g)
10
8
6
4
2
0
RIF
TOV
TOVA
Storage time (days)

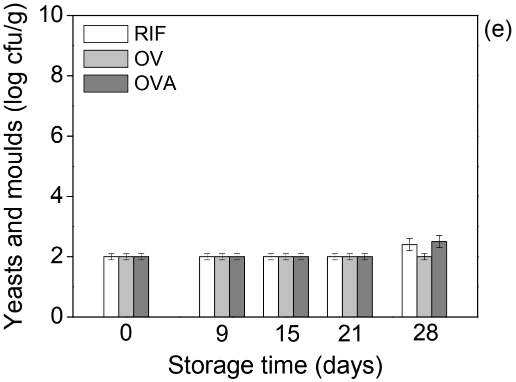


Figure 51. *Evolution of different microbial population [total viable count (a), Pseudomonas spp (b), coliforms and E. coli (c), LAB (d), yeasts and moulds (e)] of stracchino cheeses, packaged into RIF, TOV and TOVA, stored at 4°C. The acceptable limits are adopted according to Sinigaglia et al., 2008.*

In particular, the growth of *Pseudomonas* spp. (Fig. 51b) was significantly higher in stracchino cheese samples packaged into TOV and TOVA until 15 days of storage. Coliforms, yeasts and moulds grown only in the samples packaged into RIF and TOVA. Also the growth of LAB follows a particular evolution referred to the food tray used as packaging. The use of TOV as food packaging inhibited significantly the growth of LAB, moreover if compared with the samples packaged in other food trays.

For their metabolism, these bacteria are generally used as starter culture because they are able both to decrease the pH of food low enough to inhibit the growth of several microorganisms and to improve a particular sensory profile (Law et al., 1993). However, several authors have reported that non-starter LAB grow during cheese ripening and softening until about 10^7-10^8 cfu/g (McSweeney et al. 1993, Beuvier et al. 1997). Above these assumptions, it is clear that LAB inhibition reduces both the food matrix acidification and the cheese softening, according to Law et al. (1993). The rheological measurements and the results of pH evaluation on stracchino cheese samples, showed in Tab. 12, confirm our previous hypotheses. These results display that effectively the lower acidification of food matrix may influence the growth of *Pseudomonas* spp. and, as a consequence, the increase of the total viable count, according to Carraro et al. (2011). However, the lower growth of LAB seems not to be influenced by the alkaline character of TOV as previously concluded. This assumption is referred to the results showed by samples packaged in TOVA. In fact, OV and OVA films had the same alkaline character (paragraph I.3.2., p. 40), thus TOV and TOVA should present the same performances if this behaviour was due to the chemical-physical characteristics of the packaging brought in contact with the food matrix.

Table 12. *pH evolution of stracchino cheese samples stored at 4°C and packaged into RIF, TOV and TOVA. Values in the same row followed by different letter are significantly different at the p-level of 5 %.*

Packaging	0 days	9 days	15 days	21 days	28 days
RIF	5.2 ± 0.0^{a}	5.0 ± 0.1^{b}	5.0 ± 0.1^{b}	4.8 ± 0.1^{bc}	4.6 ± 0.1^{c}
TOV	5.2 ± 0.0^{a}	5.3 ± 0.1^{a}	5.2 ± 0.1^{a}	5.0 ± 0.1^{b}	5.0 ± 0.1^{b}
TOVA	5.2 ± 0.0^{a}	5.0 ± 0.0^{b}	4.9 ± 0.1^{b}	4.8 ± 0.1^{bc}	4.5 ± 0.1^{c}

Data are mean ± standard deviation

Probably, this performance against LAB may be due to other behaviours achieved by OV film and, as a consequence, by its resultant TOV food tray obtained through dry lamination process.

III.4. CONCLUSIONS

- Dry lamination process permitted to obtain, through thermoforming, two different food trays where OV and OVA films were the surface brought in contact with foodstuffs. These food trays provided higher barrier properties and experimental reproducibility.

- The amount of the initial added SP keep available at TOVA surface was lower than that available at the OVA film surface. This additional loss of SP may be ascribed to loose occurring during dry lamination process.

- The use of TOV as food packaging for mozzarella cheese has not permitted to maintain both the original colour of the cheese surface and the structural properties.

- Mozzarella cheese samples packaged into TOV showed a lower growth rate of LAB. As a result, the samples presented pH values higher than them packaged into RIF. At the same time, these samples displayed higher total viable count and the growth of *Pseudomonas* spp. equalized the limit of acceptability at 8 days of storage. The growth rate of *Pseudomonas* spp. was increased on cheese samples packaged in TOV probably because this packaging inhibited the growth of LAB, reducing the consequent acidification of the food matrix.

- TiO_2, included as white pigment in laminated OV film, was not migrated from the packaging to the mozzarella cheese conditioning brine during storage time. As a consequence, TOV can be used as food packaging for Italian mozzarella cheese.

- The use of TOV as food packaging for stracchino cheese permitted a greater maintenance of a gel-like behaviour within storage time. Contrarily, TOVA displayed the worst maintenance of the original rheological properties of the cheese.

- Also for stracchino cheese samples, TOV showed the same behaviour against LAB. Notwithstanding the same characteristics of TOV and TOVA, the latter revealed a different trend referred to the growth of LAB into the packaged cheese samples. This performance against LAB may be due to other behaviours achieved by OV film and, as a consequence, by its resultant TOV food tray obtained through dry lamination process.

PART IV

Conclusions and perspectives

IV.1. GENERAL CONCLUSIONS

- Blown film extrusion carried out in the industrial single screw extruder seemed to be a good process to obtain composite food packaging films characterized by a homogeneous matrix.

- Overall properties of produced flexible films, such as white opaque tone, alkaline character, non-absorbent and hydrophobic surfaces, were due to the composite nature of HDPE/$CaCO_3$/TiO_2 pellets. In particular, the synthetic polymer was able to influence the surface wettability, while $CaCO_3$ seemed to give both the opaque tone and the alkaline character.

- Besides whiteness and brightness, rutile TiO_2 present into the OV film seemed to accomplish a photocatalytic activity. As a consequence, under irradiation, the film was able to promote the oxidation of the organic compounds with production of CO_2 as the final degradation product. This photocatalytic property of TiO_2 embedded in the OV film was confirmed by the slight conversion of film surface wettability.

- The direct inclusion approach of an active substance inside the OV packaging material before the blown extrusion process seemed to be not a suitable way to obtain a good preservative carrier. In fact, although the resultant OVA film presented available potassium sorbate on the surface, the preservative distribution was scarcely homogeneous.

- OV films brought directly in contact with foodstuffs or their headspace permitted to maintaint the food rheological characteristics, inhibiting the growth of lactic acid bacteria. However, their scarce presence promoted the growth of other bacteria such as *Pseudomonas* spp.

- On the contrary, OVA films showed a limited effectiveness in terms of food preservation. Also presenting the same characteristics of OV films, OVA films have not permitted both the maintainance of food rheological characteristics and the inhibition of LAB.

These different performances may be due to the photocatalytic activity showed by rutile TiO_2 embedded in the film. Considering that samples were irradiated under daylight in the first 8 hours of storage, it is possible that the potassium sorbate available on the surface of food trays constituted with OVA films may be photodegradated by TiO_2, reducing their effectiveness.

IV.2. FUTURE PERSPECTIVES

- The possibility of a profitable use of OV film for food packaging applications in view of its ability to act as a wrapper capable of providing self-generated atmospheres suggests a possible increase of film properties using different crystal or size of TiO_2.

- The inhomogeneous distribution of potassium sorbate on the OVA film surface proposes the development of a technique to increase the active substance distribution and availability for specific food targets.

- Observing the inhibition of lactic acid bacteria performed by OV film, it may be interesting understand the fundamental phenomena that drive this behaviour.

- The limited effectiveness showed by OVA films suggests the opportunity to evaluate the TiO_2 photocatalytic effect on active additive.

- The results collected can be also regarded as incentives to orientate future investigations towards light irradiation aspects. For example, we could suppose a complex cycle for food preservation performed by packaging (active, opaque, heat sealed, multilayer) and light exposition (angle of light incidence, distance from light source, irradiation under innovative technologies such as pulsed light).

PART V

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ACKNOWLEDGEMENTS

Un lungo percorso come il Dottorato di Ricerca può essere considerato un piccolo compendio di vita dove le sensazioni, gli incontri, le delusioni e gli insegnamenti si susseguono quasi freneticamente, in un fragile equilibrio tra di loro. Ritengo dunque sia naturale, alla fine di un'esperienza simile, ringraziare tutte le persone che con il loro coinvolgimento diretto o indiretto hanno reso questo percorso così com'è stato.

GRAZIE

- a papà Sergio e a mamma Silvana, perchè pure immersi nelle difficoltà che la vita non ci ha mai fatto mancare non ci siamo mai disuniti e forse ci siamo uniti molto più di sempre, capendo che i silenzi, l'orgoglio e le paure non possono soffocare ciò che davvero si sente e si dovrebbe esprimere senza alcun timore, con pazienza e buonsenso;
- a Lavinia, cel mai frumos dar al acestui doctorat; ea, cu frumusetea, spiritualitatea si întelepciunea ei mi-a deschis ochii, inima si mintea, facandu-ma sa inteleg pana in strafundul meu profunzimea iubirii, fara a fi fost nevoie vreodata sa o numeasca...eu, cu tine inauntrul meu zbor...
- al Prof. Alessandro Sensidoni, per avermi proposto e dato l'opportunità di svolgere questo Dottorato come naturale prosecuzione del mio percorso di studio;
- alla Prof.ssa Rosanna Toniolo, per avermi accolto nel suo laboratorio mettendo a disposizione tutta la sua esperienza nel settore analitico;
- al Dott. Andrea Pizzariello ed al Dott. Niccolò Dossi, per la fiducia, disponibilità e competenza che hanno sempre dimostrato;
- alla Dott.ssa Donatella Peressini, per la sua disponibilità e competenza che non ha fatto mai mancare ad ogni mia necessità;
- al Prof. Franco Tubaro, per avermi accolto nel suo laboratorio e per la fiducia ed il sostegno che mi ha sempre dimostrato, non facendo mai mancare un sorriso ed una pacca sulla spalla;

- al Prof. Giuseppe Comi ed alla Dott.ssa Lucilla Iacumin, per aver coordinato tutte le analisi microbiologiche;
- ad Alberto Tomasini ed a tutti i dipendenti dell'azienda Arcadia, per la fiducia che mi hanno sempre accordato e per la disponibilità dimostrata durante questi anni di collaborazione;
- al Dott. Matteo Brazzoli ed a Sara Delfini della Sirap Gema, per la disponibilità, correttezza, professionalità e competenza che non è mai venuta meno, loro che si sono dimostrati amici prima che validi collaboratori;
- al Dott. Luigi Garavaglia, per la fiducia e considerazione sempre dimostrata ed al Dott. Marco Frusca, disponibile e competente;
- all'azienda Venchiaredo ed in particolare ai Dott. Enrico Girotto e Michele Tosato, per avermi accolto nello stabilimento produttivo e per l'aiuto illimitato che mi hanno garantito;
- al Dott. Alessandro Bacci ed ad Alvaro Cescutti di Sapio Industrie, per i suggerimenti e l'aiuto che mi hanno fornito;
- alle Dott.resse Michela Simonetti e Flavia Impellizzieri per la loro preziosa collaborazione durante la sperimentazione chimica;
- alle Dott.sse Romina Brichese e Debbie Andyanto, per il loro prezioso lavoro durante lo svolgimento delle prove microbiologiche;
- a Francesca ed Ingrid, per i loro suggerimenti durante la revisione della tesi;
- al Prof. Frederic Debeaufort ed alla Prof. Ornella Abollino per il loro competente e cortese referaggio;
- ad Alberto, il mio compagno di lavoro che per un terzo di dottorato ha condiviso le mie riflessioni, le mie idee ed i miei umori sempre con un sorriso.

Infine volevo ringraziare tutte quelle persone che durante questi 3 anni si sono celate dietro innumerevoli sorrisi da copertina intrisi di finta ingenuità per distribuire le loro parole, le loro mezze verità, i loro giudizi taglienti come se non più di lame affilate...se sono cresciuto è anche grazie a Voi...